

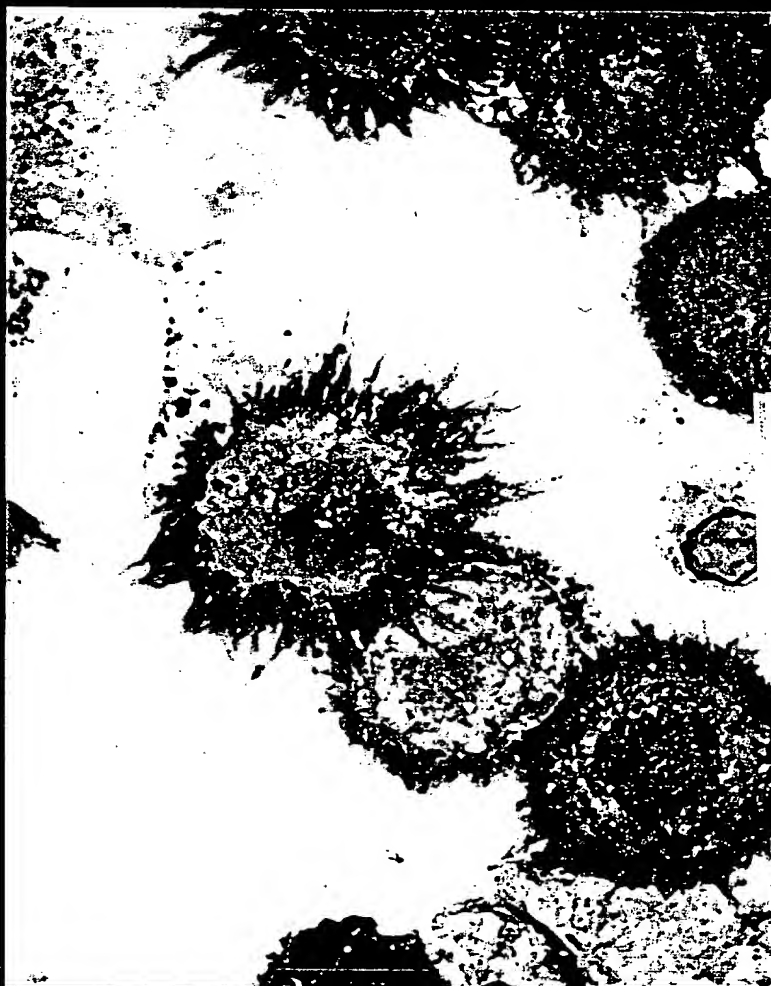
CODEN BJHDEAL

Volume 103 Number 1 October 1998

ISSN 0007-1048

British Journal of Haematology

The Official Journal of the British Society for Haematology
and the European Haematology Association
published by Blackwell Science



UNIVERSITÉ D'OTTAWA
RÉSEAU DE BIBLIOTHÈQUES

0007-1048(199810)103:1:1-0

Applicants: David J. Pinsky
U.S. Serial No.: 09/374,586
Filed: August 13, 1999
Group Art Unit: 1633

Exhibit 19

Review

- 1 Hepatitis in haemophilia
Paul L. F. Giangrande

Platelets, haemostasis and thrombosis

RESEARCH PAPERS

- 10 Major surgery seems not to influence HIV disease progression in haemophilia patients
Jan Astermark, Thomas Löfqvist, Sam Schulman, Lennart Stigendal, Stefan Lethagen, Inga Marie Nilsson and Erik Berntorp
- 15 Small GTP-binding in human endothelial cells
Hubert P. J. C. de Leeuw, Pauline M. Koster, Jero Calafat, Hans Janssen, Anton-Jan van Zonneveld, Jan A. van Mourik and Jan Voorberg
- 20 Studies of multimerin in patients with von Willebrand disease and platelet von Willebrand factor deficiency
Christine I. Chen, Augusto B. Federici, Elisabeth M. Cramer, Maria T. Canciani, Paul Harrison, Shilun Zheng, Jean-Marc Massé, Pier M. Mannucci and Catherine P. M. Hayward
- 29 Mechanisms involved in the antiplatelet activity of *Escherichia coli* lipopolysaccharide in human platelets
J. R. Sheu, W. C. Hung, Y. C. Kan, Y. M. Lee and M. H. Yen

SHORT REPORTS

- 39 Type 2N von Willebrand disease due to Arg91Gln substitution and a cytosine deletion in exon 18 of the von Willebrand factor gene
A. Casonato, C. Gaucher, E. Pontara, A. Zucchetto, P. Zerbinati, C. Mazurier and A. Girolami
- 42 Effect of the MTHFR C677T variant on risk of venous thromboembolism: interaction with factor V Leiden and prothrombin (F2G20210A) mutations
Karen Brown, Roger Luddington and Trevor Baglin

RESEARCH PAPERS

- 45 Molecular, haematological and clinical studies of a silent β -gene C→G mutation at 6 bp 3' to the termination codon (+1480 C→G) in twelve Greek families
E. Maragoudaki, C. Vrettou, E. Kanavakis, T. Treager-Synodinos, A. Metaxotou-Mavrommati and C. Kattamis
- 52 Status of *Plasmodium falciparum* towards catalase
G. Clarebout, C. Slomianny, P. Delcourt, B. Leu, A. Masset, D. Camus and D. Dive

Haematological malignancy

RESEARCH PAPERS

- 60 Minimal residual disease in childhood acute lymphoblastic leukaemia quantified by aspirate and trephine: is the disease multifocal?
P. J. Sykes, M. J. Brisco, E. Hughes, L. E. Snell, G. Dolman, S-H. Neoh, L-M. Peng, I. Toogood, W. N. Venables and A. A. Morley
- 66 Activating point mutations in the β_c chain of the GM-CSF, IL-3 and IL-5 receptors are not a major contributory factor in the pathogenesis of acute myeloid leukaemia
Robin W. Freeburn, Rosemary E. Gale and David C. Linch
- 72 Detection of karyotypic aberrations in acute myeloblastic leukaemia: a prospective comparison between PCR/FISH and standard cytogenetics in 140 patients with *de novo* AML
Jürgen Krauter, Wolfgang Peter, Ulrich Pascheberg, Barbara Heinze, Lothar Bergmann, Dieter Hoelzer, Michael Lübbert, Günther Schlimok, Renate Arnold, Hartmut Kirchner, Maria Port, Arnold Ganser and Gerhard Heil
- 79 Gamma-irradiation does not impair ATRA-induced maturation of myeloid leukaemic cells: implication for combined radiation and differentiation therapy
Thi My Anh Neildez-Nguyen, Alain Chapel, Michel Arock, Jérôme Vétillard and Dominique Thierry
- 87 Identification of transcription factors expressed during ATRA-induced neutrophil differentiation of HL60 cells
K. I. Mills, V. Walsh, A. E. Gilkes, L. J. Woodgate, G. Brown and A. K. Burnett

- 93 Cell lineage specific involvement in acute promyelocytic leukaemia (APL) using a combination of May-Grünwald-Giemsa staining and fluorescence *in situ* hybridization techniques for the detection of the translocation t(15;17)(q22;q12)
Torsten Haferlach, Helmut Löffler, Christina Nickenig, Lotte Ramm-Petersen, Marlies Meeder, Robert Schoch, Brigitte Schlegelberger, Susanne Schnittger, Claudia Schoch and Wolfgang Hiddemann
- 100 A systematic collaborative overview of randomized trials comparing idarubicin with daunorubicin (or other anthracyclines) as induction therapy for acute myeloid leukaemia
The AML Collaborative Group
- 110 Expression and functional role of urokinase-type plasminogen activator receptor in normal and acute leukaemic cells
F. Lanza, G. L. Castoldi, B. Castagnari, R. F. Todd III, S. Moretti, S. Spisani, A. Latorraca, E. Focarile, M. G. Roberti and S. Traniello
- 124 Quantitative and qualitative alterations of long-term culture-initiating cells in patients with acute leukaemia in complete remission
M. Yao, S. Bouchet, C. Harnois, M. C. Giarratana, J. P. Laporte, N. C. Gorin, M. Lopez and L. Douay
- 129 Paediatric Hodgkin's disease in Spain: association with Epstein-Barr virus strains carrying latent membrane protein-1 oncogene deletions and high frequency of dual infections
Almudena Santón, Carmen Martín, Ana Isabel Manzanal, María Victoria Preciado and Carmen Bellas
- 137 *In vitro* growth in acute myeloblastic leukaemia: relationship with other clinico-biological characteristics of the disease
M. C. del Cañizo, A. Brufau, J. Almeida, J. Galende, M. A. García Marcos, R. García, J. Fernández Calvo, F. Ramos, P. Fisac, A. Orfao and J. E. San Miguel
- 143 Patterns of cytokine expression in AIDS-related non-Hodgkin's lymphoma
Cristina Pastore, Gianluca Gaidano, Paolo Ghia, Lucia Fassone, Anna Maria Cilia, Annunziata Gloghini, Daniela Capello, Daniela Buonaiuto, Silvana Gonella, Silvio Roncella, Antonio Carbone and Giuseppe Saglio
- 150 Serological evidence of an association between chlamydial infections and malignant lymphomas
T. I. Anttila, T. Lehtinen, M. Leinonen, A. Bloigu, P. Koskela, M. Lehtinen and P. Saikku
- 157 B-cell chronic lymphocytic leukaemia with CD8 expression: report of 10 cases and immunochemical analysis of the CD8 antigen
S. P. Mulligan, L. P. Dao, S. E. Francis, M. E. Thomas, J. Gibson, M. F. Cole-Sinclair and M. Wolf
- 163 IL-4 improves the detection of cytogenetic abnormalities in multiple myeloma and increases the proportion of clonally abnormal metaphases
J. M. Hernández, N. C. Gutiérrez, J. Almeida, J. L. García, M. A. Sánchez, G. Mateo, A. Ríos and J. E. San Miguel
- 168 Transcriptional silencing of the p16 gene in human myeloma-derived cell lines by hypermethylation
Ivy H. N. Wong, Margaret H. L. Ng, Joseph C. K. Lee, Kwok-Wai Lo, Yuk-Fei Chung and Dolly P. Huang
- 176 A role for tumour necrosis factor- α , Fas and Fas-Ligand in marrow failure associated with myelodysplastic syndrome
Geoffrey M. Gersuk, Cassandra Beckham, Michael R. Loken, Peter Kiener, Jeanne E. Anderson, Alan Farrand, Anthony B. Troutt, Jeffrey A. Ledbetter and H. Joachim Deeg
- 189 Exposure to myelotoxic agents and myelodysplasia: case-control study and correlation with clinicobiological findings
Gian Matteo Rigolin, Antonio Cuneo, Maria Grazia Roberti, Antonella Bardi, Renato Bigoni, Nadia Piva, Claudia Minotto, Paola Agostini, Cristiano De Angeli, Laura Del Senno, Romedio Spanedda and Gianluigi Castoldi
- 198 A comparative analysis of lipid-complexed and liposomal amphotericin B preparations in haematological oncology
A. D. Clark, S. McKendrick, P. J. Tansey, I. M. Franklin and R. Chopra
(Corrigendum p. 1215)
- 205 Liposomal amphotericin B compared with amphotericin B deoxycholate in the treatment of documented and suspected neutropenia-associated invasive fungal infections
Alexander C. A. P. Leenders, Simon Daenen, Rob L. H. Jansen, Wim C. J. Hop, Bob Lowenberg, Pierre W. Wijermans, Jan Cornelissen, Raoul Herbrecht, Hans van der Lelie, Henk C. Hoogsteden, Henri A. Verbrugh and Siem de Marie

SHORT REPORTS

- 213 **Cytogenetic and molecular study of 32 Down syndrome families: potential leukaemia predisposing role of the most proximal segment of chromosome 21q**
Simona Cavani, Chiari Perfumo, Alessandra Argusti, Mauro Pierluigi, Lucia Perroni, Kjeld Schmiegelow, Michael B. Petersen, Finbarr E. Cotter, Paolo Strigini, Franca Dagna-Bricarelli and Dean Nizetić
- 217 **Unbalanced expression of Fas and CD40 in mantle cell lymphoma**
Katharina Clodi, Virginia Snell, Shourong Zhao, Fernando Cabanillas, Michael Andreeff and Anas Younes
- 220 **Conservative management of follicular non-Hodgkin's lymphoma in childhood**
A. Atrá, S. T. Meller, R. S. Stevens, R. Hobson, R. Grundy, R. L. Carter and C. R. Pinkerton
- 224 **A polymorphism in exon b2 of the major breakpoint cluster region (M-ber) identified in chronic myeloid leukaemia patients**
Rosely de V. Meissner, Paula M. B. Dias, Dimas T. Covas, Fani Job, Márcia Leite and Nance B. Nardi

Transplantation

RESEARCH PAPERS

- 227 **Mobilization and transplantation of Philadelphia chromosome-negative peripheral blood progenitor cells in patients with CML**
Cornelius F. Waller, Monika Heinzinger, Antonia Rosenstiel and Winand Lange
- 235 **Factors associated with successful mobilization of peripheral blood progenitor cells in 200 patients with lymphoid malignancies**
Nicolas Ketterer, Gilles Salles, Isabelle Moullet, Charles Dumontet, Assia ElJaafari-Corbin, Pierre Tremisi, Catherine Thieblemont, Brigitte Durand, Eve-Marie Neidhardt-Berard, Hanadi Samaha, Dominique Rigal and Bertrand Coiffier
- 243 **Unusual complications after bone marrow transplantation for dyskeratosis congenita**
Vanderson Rocha, Agnès Devergie, Gerard Socié, Patricia Ribaud, Hélène Espérou, Nathalie Parquet and Eliane Gluckman
- 249 **Transplantation for Fanconi's anaemia: long-term follow-up of fifty patients transplanted from a sibling donor after low-dose cyclophosphamide and thoraco-abdominal irradiation for conditioning**
Gérard Socié, Agnès Devergie, Théodore Girinski, Gaëlle Piel, Patricia Ribaud, Hélène Espérou, Nathalie Parquet, Odile Maarek, Maria-Helena Noguera, Patrice Richard, Olivier Brison and Eliane Gluckman

SHORT REPORT

- 256 **Apparent reactivation of a red cell alloantibody in a healthy individual after G-CSF administration**
F. Norol, P. Bonin, F. Charpentier, P. Bierling, F. Beaujean, J. P. Cartron, D. Bories and M. Kuentz

Transfusion medicine

RESEARCH PAPER

- 259 **Effect of 1-β-D-arabino-furanosyl-cytosine (ara-C) induction of K562 cells on expression of Rh and other blood group active proteins**
Edith Wiener, Allan Shiels, Sunitha N. Wickramasinghe and Neil D. Avent

SHORT REPORT

- 268 **Delayed haemolytic transfusion reaction due to anti-M antibody**
Juan Manuel Sancho, Misericordia Pujol, Francesc Fernández, Manuel Soler, Pedro Manzano and Evarist Feliu

Rapid Papers

- 270 **Coexpression of CD40 and CD40 ligand in B-cell lymphoma cells**
Katherina Clodi, Zahra Asgary, Shourong Zhao, Kay-Oliver Kliche, Fernando Cabinallas, Michael Andreeff and Anas Younes
- 276 **Increased expression of phosphatidylinositol-specific phospholipase C resistant prion proteins on the surface of activated platelets**
Karel Holada, Tracy Heath Mondoro, Jacqueline Muller and Jaroslav G. Vostal

Correspondence

- 283 **The current use of low molecular weight heparins in clinical medicine**
Karen Phekoo, Steve Schey and Beverley J. Hunt
- 284 **Successful donor lymphocyte infusion (DLI) in a patient with myelodysplastic syndrome (MDS) after failure of T-cell-depleted bone marrow transplantation (TD-BMT)**
L. Castagna, A. El Weshii, J. H. Bourhis, V. Ribrag, P. Naccache, J. M. Vantelon, P. Brault and J. L. Pico

- 286 **Book Review**

Review

- 287 **Molecular genetics and Fanconi anaemia: new insights into old problems**
A. A. Clarke, J. C. W. Marsh,
E. C. Gordon-Smith and T. R. Rutherford

Bone marrow hypoplasia

RESEARCH PAPER

- 297 **Long-term administration of G-CSF for aplastic anaemia is closely related to the early evolution of monosomy 7 MDS in adults**
Ken Kaito, Masayuki Kobayashi,
Toshio Katayama, Hidekazu Masuoka,
Takaki Shimada, Kaichi Nishiwaki,
Toru Sekita, Hiroko Otsubo,
Yojiu Ogasawara and Tatsuo Hosoya

SHORT REPORTS

- 304 **Phase I/II trial of PIXY321 (granulocyte-macrophage colony stimulating factor/interleukin-3 fusion protein) for treatment of inherited and acquired marrow failure syndromes**
Douglas S. Taylor, Yisheng Lee, Colin A. Sieff,
Alan Homans, Leslie Garrison and Eva C. Guinan
- 308 **Th1/Th2 balance alteration in the clinical course of a patient with pure red cell aplasia and thymoma**
Shoji Fujisao and Hiroyuki Tsuda
- 311 **Mutational screening of thrombopoietin receptor gene (c-mpl) in patients with congenital thrombocytopenia and absent radii (TAR)**
Pierluigi Strippoli, Anna Savoia,
Achille Iolascon, Roberto Tonelli,
Maria Savino, Paola Giordano,
Michele D'Avanzo, Fausta Massolo,
Franco Locatelli, Caterina Borgna,
Domenico De Mattia, Leopoldo Zelante,
Guido Paolucci and Gian Paolo Bagnara
- 315 **Pregnancy and severe aplastic anaemia: causal relation or coincidence?**
H. M. Oosterkamp, A. Brand,
J. C. Kluin-Nelemans and
J. P. Vandenbroucke

Haemopoiesis

RESEARCH PAPERS

- 317 **pH is a potent modulator of erythroid differentiation**
Todd A. McAdams, William M. Miller
and E. Terry Papoutsakis

- 326 **In utero transplantation of human fetal haemopoietic cells in NOD/SCID mice**
Curtis W. Turner, David R. Archer,
John Wong, Andrew M. Yeager
and William H. Fleming

- 335 **Enhanced human cell engraftment in mice deficient in RAG2 and the common cytokine receptor γ chain**
Jacki P. Goldman, Michael P. Blundell,
Lucien Lopes, Christine Kinnon,
James P. Di Santo and Adrian J. Thrasher

- 343 **All-trans-retinoic acid up-regulates CD38 but not c-Kit antigens on human marrow CD34⁺ cells without recruitment into cell cycle**
Olivier Herault, Jorge Domenech,
Michel Degennie, Jean-Louis Bremond,
Luc Sensebe, Marie-Christine Bernard,
Christian Binet and Philippe Colombat

- 351 **Differential development of fetal and adult haemoglobin profiles in colony culture: isolation of fetal nucleated red cells by two-colour fluorescence labelling**
Ralph M. Bohmer, DongKai Zhen
and Diana W. Bianchi

Iron and erythrocytes

RESEARCH PAPERS

- 361 **Combined therapy with deferiprone and desferrioxamine**
B. Wonke, C. Wright and A. V. Hoffbrand
- 365 **Alpha thalassaemia is associated with increased soluble transferrin receptor levels**
D. C. Rees, T. N. Williams, K. Maitland,
J. B. Clegg and D. J. Weatherall
- 370 **A case of non- β -globin gene linked β thalassaemia in a Dutch family with two additional α -gene defects: the common $-\alpha^{37}$ deletion and the rare IVS1-116 (A \rightarrow G) acceptor splice site mutation**
P. C. Giordano, C. L. Harteveld, H. L. Haak,
D. Batelaan, P. van Delft, R. J. Plug, M. Emonts,
R. Zanardini and L. F. Bernini
- 377 **Molecular characterization of the PK-LR gene in pyruvate kinase deficient Spanish patients**
R. Zarza, R. Alvarez, A. Pujades, B. Nomdedeu,
A. Carrera, J. Estella, A. Remacha, J. M. Sánchez,
M. Morey, T. Cortes, G. Pérez Lungmus,
E. Beuro and J. L. Vives Corrons

SHORT REPORT

- 383 **A genetic syndrome associating dehydrated hereditary stomatocytosis, pseudohyperkalaemia and perinatal oedema**
S. Grootenboer, P. O. Schischmanoff, T. Cynober,
J.-C. Rodrigue, J. Delaunay, G. Tchernia
and J.-P. Dommergues

Leucocytes

RESEARCH PAPER

- 387 Effects of r-metHuG-CSF on polymorphonuclear leucocyte kinetics and function in patients on continuous ambulatory peritoneal dialysis
J. Turzanski, S. P. M. Crouch, M. Andrews, M. Rose, R. Finch, R. Burden, M. Holliday and J. Fletcher

Platelets, haemostasis and thrombosis

RESEARCH PAPERS

- 397 Relationships between severe neonatal thrombocytopenia and maternal characteristics in pregnancies associated with autoimmune thrombocytopenia
Anne Sylvie Valat, Marie Thérèse Caulier, Patrick Devos, Lucia Rugeri, Bénédicte Wibaut, Pascal Vaast, Francis Puech, Francis Bauters and Brigitte Jude
- 402 Inhibition of platelet activation by the Alzheimer's disease amyloid precursor protein
Anna Henry, Qiao-Xin Li, Denise Galatis, Lars Hesse, Gerd Multhaupt, Konrad Beyreuther, Colin L. Masters and Roberto Cappai
- 416 Anti-endothelial cell antibodies in primary antiphospholipid syndrome and SLE: patterns of reactivity with membrane antigens on microvascular and umbilical venous cell membranes
M. B. Hill, J. L. Phipps, P. Hughes and M. Greaves

SHORT REPORTS

- 422 Analysis of risk factors for over-anticoagulation in patients receiving long-term warfarin
Sabapathy Panneerselvam, Caroline Baglin, Wendy Lefort and Trevor Baglin
- 425 Splicing and missense mutations in the human FXIII gene causing FXIII deficiency: effects of these mutations on FXIII RNA processing and protein structure
Rashida Anwar, Louise Gallivan, Krzysztof J. A. Miloszewski and Alexander F. Markham

Transfusion medicine

RESEARCH PAPERS

- 429 Heterogeneity of blood group RhE variants revealed by serological analysis and molecular alteration of the *RHCE* gene and transcript
France Noizat-Pirenne, Isabelle Mouro, Pierre Gane, Yasuto Okubo, Yasuto Hori, Philippe Rouger, Pierre-Yves Le Pennec and Jean-Pierre Cartron

- 437 Human platelet antigen genotyping using a fluorescent SSCP technique with an automatic sequencer
André Quintanar, Vincent Jallu, Yann Legros and Cécile Kaplan

SHORT REPORT

- 445 Bioactive substances in buffy-coat-derived platelet pools stored in platelet-additive solutions
Lisbeth Edvardsen, Ellen Taaning, Tommie Mynster, Jesper Hvolris, Ole Drachman and Hans Jorgen Nielsen

Gene transfer

RESEARCH PAPER

- 449 Rapid retroviral infection of human haemopoietic cells of different lineages: efficient transfer in fresh T cells
Martino Introna, Anna Maria Barbui, Josée Golay, Federica Bambacioni, Raffaella Schirò, Sergio Bernasconi, Ferruccio Breviario, Eugenio Erba, Gianmaria Borleri, Tiziano Barbui, Andrea Biondi and Alessandro Rambaldi

Haematological malignancy

RESEARCH PAPERS

- 462 Analysis of mutations at the DNA repair genes in acute childhood leukaemia
Ying-Wei Lin, Masaru Kubota, Seiji Koishi, Machiko Sawada, Ikuya Usami, Ken-Ichiro Watanabe and Yuichi Akiyama
- 467 Mutation of p16, p21 or cyclin dependent kinase 4 is rare in acute lymphoblastic leukaemia
Mohammed H. Qari, Salim H. Khalil, Marios Kambouris and Brian F. Meyer
- 473 Cryptic deletions and inversions of chromosome 21 in a phenotypically normal infant with transient abnormal myelopoiesis: a molecular cytogenetic study
Helena M. Kempfski, Janet L. Craze, Judith M. Chessells and Brian R. Reeves
- 480 Influence of SDZ-PSC833 on daunorubicin intracellular accumulation in bone marrow specimens from patients with acute myeloid leukaemia
Jean-Louis Merlin, Agnès-Paule Guerci, Sophie Marchal, Corinne Bour, Pascal Colosetti, Agapi Katakaki and Olierio Guerci

- 488 Indolent course as a relatively frequent presentation in T-prolymphocytic leukaemia
Richard Garand, Jean Goasguen, André Brizard, Jacqueline Buisine, Agnès Charpentier, Jean François Claisse, Eliane Duchayne, Monique Lagrange, Christine Segonds, Xavier Troussard and Georges Flandrin

- 495 FISH detection of chromosome 14q32/IgH translocations: evaluation in follicular lymphoma
Katrina A. Rack, Florence Salomon-Nguyen, Isabelle Radford-Weiss, Maria Ocana Gil, Christian Schmitt, Coralie Belanger, Sylvie Nusbaum, Michel Vekemans, Françoise Valensi and Elizabeth A. Macintyre

- 505 Splenic irradiation for symptomatic splenomegaly associated with myelofibrosis with myeloid metaplasia
Michelle A. Elliott, Michael G. Chen, Murray N. Silverstein and Ayalew Tefferi

- 512 VAD or VMBCP in multiple myeloma refractory to or relapsing after cyclophosphamide-prednisone therapy (protocol MY 85)
Ph. Mineur, J. F. Ménard, X. Le Loët, J. F. Bernard, B. Grosbois, J. P. Pollet, I. Azais, J. Ph. Laporte, C. Doyen, A. De Gramont, M. Wetterwald, L. Euler-Ziegler, A. M. Peny, M. Monconduit and J. L. Michaux

- 518 Human myeloma cell apoptosis induced by interferon- α
Takemi Otsuki, Osamu Yamada, Haruko Sakaguchi, Akiko Tomokuni, Hideho Wada, Yoshihito Yawata and Ayako Ueki

SHORT REPORTS

- 530 Anti-myeloma activity of pamidronate *in vivo*
Madhav V. Dhodapkar, Joginder Singh, Jayesh Mehta, Athanasios Fassas, K. R. Desikan, Martin Perlman, Nikhil C. Munshi and Bart Barlogie

- 533 Ifosfamide and vinorelbine: an active regimen for patients with relapsed or refractory Hodgkin's disease
V. Bonfante, S. Viviani, A. Santoro, L. Devizzi, A. Di Russo, M. Zanini, F. Soncini, H. Soto Parra, P. Valagussa and G. Bonadonna

- 536 The ATM gene and susceptibility to childhood T-cell acute lymphoblastic leukaemia
Seisho Takeuchi, Michiaki Koike, Susan Park, Taku Seriu, Claus R. Bartram, Harry E. Taub, Ian K. Williamson, Jeffrey Grewal, Hirokuni Taguchi and H. Phillip Koefler

- 539 Oncogenesis *in utero*: fetal death due to acute myelogenous leukaemia with an MLL translocation
Stephen P. Hunger, Loris McGavran, Lynne Meltesen, Natalie B. Parker, C. Kenneth Kassenbrock and Mitchell A. Bitter

Transplantation

RESEARCH PAPERS

- 543 Prevention of EBV-induced B-lymphoproliferative disorder by *ex vivo* marrow B-cell depletion in HLA-phenotypical or non-identical T-depleted bone marrow transplantation
M. Cavazzano-Calvo, D. Bensoussan, N. Jabado, E. Haddad, E. Yvon, M. Moskwa, A. Tachet des Combes, M. Buisson, P. Morand, J. M. Virion, F. Le Deist and A. Fischer

- 552 Haemopoietic cell transplantation in children with juvenile myelomonocytic leukaemia
M. L. MacMillan, S. M. Davies, P. J. Orchard, N. K. C. Ramsay and J. E. Wagner

- 559 Salvage of patients with acute promyelocytic leukaemia with residual disease following ABMT performed in second CR using all-*trans* retinoic acid
David Grimwade, Rahman Jamal, Nicholas Goulden, Helena Kempski, Stefano Mastrangelo and Paul Veys

- 563 Successful adoptive immunotherapy for relapse of AML 9 years after T-cell-depleted BMT
Hartmut Bertz, Regine Kunzman, Donald Bunjes and Jürgen Finke

Rapid Papers

- 565 Evidence of cytogenetic and molecular remission by allogeneic cells after immunosuppressive therapy alone
Angelo M. Carella, Enrica Lerma, Maria T. Corsetti, Anna Dejana, Lidia Celesti, Lucia Casarino, Francesco De Stefano and Francesco Frassoni
- 568 Primordial role of CD34⁺38⁻ cells in early and late trilineage haemopoietic engraftment after autologous blood cell transplantation
Ph. Hénon, H. Sovalat, M. Becker, Y. Arkam, M. Ojeda-Urbe, J. P. Raidot, F. Hussein, E. Wunder, D. Bourderont and B. Audhuy

Correspondence

- 582 Bone marrow donation without consent?
M. M. Reid
Reply: S. E. Mumford and A. Oakhill

- 583 **Antithrombin concentrate alone may not prevent venous thromboembolism**
I. O. Singer, A. Sefcick, R. C. Tait, K. W. Lindsay and I. D. Walker
- 584 **Vitamin K2 combined with all-trans retinoic acid induced complete remission of relapsing acute promyelocytic anaemia**
Hiroshi Fujita, Junji Tomiyama and Takehiko Tanaka
- 585 **Sampling bias and logistical problems of molecular analyses**
Martin F. Fey, Margrith Blaser, Martin Wernli, Martine Jotterand, Rudolf Maibach, Alois Gratwohl and Andreas Tobler
- 587 **Hydroxyurea treatment in thalassaemia**
K. Konstantopoulos and S. Lymperi
- 588 **APC resistance in recurrent fetal loss in the Indian population**
Jyoti Kotwal, Renu Saxena, Sujata Mohanty and Deepika Deka
- 589 **Book Reviews**
- 590 **Announcements**

VOL. 103 NO 3

DECEMBER-I 1998

Annotation

- 591 **Telomeres and haemopoiesis**
Robert F. Wynn, Michael A. Cross and Nydia G. Testa

Review

- 594 **Developments in allogeneic peripheral blood progenitor cell transplantation**
N. H. Russell, A. Gratwohl and N. Schmitz

Transplantation**RESEARCH PAPERS**

- 601 **Composition and function of peripheral blood stem and progenitor cell harvests from patients with severe active rheumatoid arthritis**
J. A. Snowden, V. Nink, M. Cooley, J. Zaunders, M. Keir, L. Wright, S. T. Milliken, P. M. Brooks and J. C. Biggs
- 610 **Evaluation of breast tumour cell contamination in the bone marrow and leukapheresis collections by RT-PCR for cytokeratin-19 mRNA**
Alessandro M. Vannucchi, Alberto Bosi, Stephanie Glinz, Paolo Pacini, Silvia Linari, Riccardo Saccardi, Renato Alterini, Luigi Rigacci, Stefano Guidi, Letizia Lombardini, Giovanni Longo, Maria P. Mariani and Pierluigi Rossi-Ferrini

- 618 **Extensive phenotypic analysis of CD34 subsets in successive collections of mobilized peripheral blood progenitors**
Lucien Perey, Rowayda Peters, Sandro Pampallona, Philippe Schneider, Nicole Gross and Serge Leyvraz
- 630 **Bone marrow transplantation for adults with acute leukaemia and 11q23 chromosomal abnormalities**
D. L. Forrest, T. J. Nevill, D. E. Horsman, D. A. Brockington, H. C. Fung, C. L. Toze, E. A. Conneally, D. E. Hogge, H. J. Sutherland, S. H. Nantel, J. D. Shepherd and M. J. Barnett
- 639 **Expansion of megakaryocyte progenitors from human umbilical cord blood using a new two-step separation procedure**
Marjorie Pick, Arnon Nagler, Dan Grisaru, Amiram Eldor and Varda Deutsch

SHORT REPORT

- 651 **Autologous bone marrow transplantation for non-Hodgkin's lymphoma resulting in long-term remission of coincidental Crohn's disease**
Ashwin Kashyap and Stephen J. Forman

Haematological malignancy**RESEARCH PAPERS**

- 653 **Seasonal variation in the incidence of Hodgkin's disease**
Stuart Douglas, Mario Cortina-Borja and Ray Cartwright
- 663 **In vivo immune responses to idiotypic VH complementarity-determining region 3 peptide vaccination in B-cell non-Hodgkin's lymphoma**
Yue-Jin Wen and Seah H. Lim
- 669 **Establishment of an IL-2-dependent cell line derived from 'nasal-type' NK/T-cell lymphoma of CD2⁺, sCD3⁻, CD3ε⁺, CD56⁺ phenotype and associated with the Epstein-Barr virus**
Yoshitoyo Kagami, Shigeo Nakamura, Ritsuro Suzuki, Shinsuke Iida, Yasushi Yatabe, Yasutaka Okada, Tomoko Kobayashi, Tasuya Tsurumi, Masao Seto, Michinori Ogura, Osamu Taguchi and Yasuo Morishima
- 678 **Paclitaxel plus high-dose cyclophosphamide with G-CSF support in patients with relapsed and refractory aggressive non-Hodgkin's lymphoma**
Anas Younes, Jorge Romaguera, Ofelia Mesina, Frederick Hagemeister, Andreas H. Sarris, Maria A. Rodriguez, Peter McLaughlin, Hector A. Preti, Carlos Bachier and Fernando Cabanillas

- 684 **CD40 regulation of death domain containing receptors and their ligands on lymphoma B cells**
Patricia Ribeiro, Nathalie Renard,
Krzysztof Warzocha, Carole Charlot,
Lorena Jeandenant, Evelyne Callet-Bauchu,
Bertrand Coiffier and Gilles Salles
 - 690 **Bolus administration of cladribine in the treatment of Waldenström macroglobulinaemia**
Eva S. Liu, Carol Burian, William E. Miller
and Alan Saven
 - 696 **Anticipation in familial plasma cell dyscrasias**
Hari A. Deshpande, Xiao-ping Hu,
Patricia Marino, Naveed A. Jan
and Peter H. Wiernik
 - 704 **Chromosomal abnormalities
in systemic amyloidosis**
Rafael Fonseca, Gregory J. Ahmann, Syed M. Jalal,
Gordon W. Dewald, Dirk R. Larson,
Terry M. Therneau, Morie A. Gertz,
Robert A. Kyle and Philip R. Greipp
 - 711 **Expression of p210 and p190 BCR-ABL
due to alternative splicing
in chronic myelogenous leukaemia**
Brian D. Lichty, Armand Keating,
Jeannie Callum, Karen Yee,
Ruth Croxford, George Corpus,
Bevoline Nwachukwu, Peter Kim,
Joyce Guo and Suzanne Kamel-Reid
 - 716 **The susceptibility of Philadelphia chromosome
positive cells to FAS-mediated apoptosis
is not linked to the tyrosine kinase activity
of BCR-ABL**
Joanna Gora-Tybor, Michael W. M. Deininger,
John M. Goldman and Junia V. Melo
 - 721 **Arsenic trioxide inhibits growth of human
T-cell leukaemia virus type I infected T-cell lines
more effectively than retinoic acids**
Kenji Ishitsuka, Shuichi Hanada,
Shinsuke Suzuki, Atae Utsonomiya,
Yoshiko Chyuman, Syogo Takeuchi,
Taketsugu Takeshita, Sigemi Shimotakahara,
Kimiharu Uozumi, Torahiko Makino
and Terukatsu Arima
 - 729 **Ultrastructural characteristics and lysozyme
content in hypergranular and variant type
of acute promyelocytic leukaemia**
Dina Shaft, Mordechai Shtalrid, Alain Berebi,
Daniel Catovsky and Peretz Resnitzky
 - 740 **Systemic mastocytosis associated with acute
myeloid leukaemia: report of two cases and
detection of the *c-kit* mutation Asp-816 to Val**
W. R. Sperr, S. Walchshofer, H.-P. Horny,
M. Födinger, I. Simonitsch, R. Fritsche-Polanz,
I. Schwarzingger, E. Tschachler, C. Sillaber,
W. Hagen, K. Geissler, A. Chott, K. Lechner
and P. Valent
 - 750 **Near-haploid common acute lymphoblastic
leukaemia of childhood with a second hyperdiploid
line: a DNA ploidy and fluorescence *in-situ*
hybridization study**
S. K. Ma, G. C. F. Chan, T. S. K. Wan, C. K. Lam,
S. Y. Ha, Y. L. Lau and L. C. Chan
 - 756 **Longitudinal observation and outcome of
nonfamilial childhood haemophagocytic
syndrome receiving etoposide-containing
regimens**
Jiann-Shiuh Chen, Kai-Hsin Lin,
Dong-Tsamn Lin, Rong-Long Chen,
Shiann-Tarng Jou and Ih-jen Su
 - 763 **The *in-vitro* generation of dendritic cells
from blast cells in acute leukaemia**
S. P. Robinson, N. English, R. Jaju, L. Kearney,
S. C. Knight and C. D. L. Reid
 - 772 **No treatment for low-risk thrombocythaemia:
results from a prospective study**
M. Ruggeri, G. Finazzi, A. Tosetto, S. Riva,
F. Rodeghiero and T. Barbui
 - 778 **Plasma thrombopoietin (TPO) levels and
expression of TPO receptor on platelets in patients
with myelodysplastic syndromes**
H. Tamura, K. Ogata, S. Luo, K. Nakamura,
N. Yokose, K. Dan, K. Tohyama, Y. Yoshida,
H. Hamaguchi, H. Sakamaki, T. Kuwaki,
T. Tahara, T. Kato and T. Nomura
- SHORT REPORTS
- 785 **Poor response rate to a continuous schedule of
Amifostine therapy for 'low/intermediate risk'
myelodysplastic patients**
D. T. Bowen, C. Denzlinger, W. Brugger,
D. Culligan, K. Gelly, S. Adlakha, M. Groves,
M. Hepburn and L. Kanz
 - 788 **Reactivation of Behçet's disease in the course
of multicentric HHV8-positive Castleman's
disease: long-term complete remission
by a combined chemo/radiation and
interferon- α therapy regimen**
Robert Strohal, Erwin Tschachler, Stefan Breyer,
Aumed Uthman, Ingrid Simonitsch,
Sigfried Trating, Werner Scheithauer,
Georg Stingl and Gabriela V. Kornek
 - 791 **A novel BCR-ABL fusion gene (e2/1a)
in a patient with Philadelphia-positive
chronic myelogenous leukaemia
and an aggressive clinical course**
J. L. Byrne, G. I. Carter, J. M. Davies,
A. P. Haynes, N. H. Russell
and N. C. P. Cross
 - 795 **Cerebral candidiasis in a child
1 year after leukaemia**
Deborah A. Tweddle, J. Clive Graham,
Gillian S. Shankland and Jennifer Kernahan

Leucocytes

RESEARCH PAPERS

- 798 **Mast cell recruitment after subcutaneous injection of RANTES in the sole of the rat paw**
Pio Conti, Marcella Reale,
Renato C. Barbacane, Mario Felaco,
Alfredo Grilli and Theoharis C. Theoharides
- 804 **Effect of the interaction between fibronectin and VLA-4 on the proliferation of human B cells, especially a novel human B-cell line, OPM-3**
Hitoshi Yoshida, Tetsuo Nishiura,
Takahiro Karasuno, Itaru Matsumura,
Jun Ishikawa, Masafumi Yoshimura,
Takafumi Yokota, Yu Okajima,
Megumu Ogawa, Yuzuru Kanakura,
Yoshiaki Tomiyama and Yuji Matsuzawa

Erythrocytes and iron

RESEARCH PAPERS

- 813 **The haemochromatosis mutations do not modify the clinical picture of thalassaemia major in patients regularly transfused and chelated**
Caterina Borgna-Pignatti,
Agostina Solinas, Cristina Bombieri,
Rocco Micciolo, Maria Rita Gamberini,
Piero De Stefano, Ernesto De Menis
and Pier Franco Pignatti
- 817 **Iron status in pregnant women: which measurements are valid?**
N. R. van den Broek, E. A. Letsky,
S. A. White and A. Shenkin
- 825 **Effects of four species of interferon- α on cultured erythroid progenitors from congenital dyserythropoietic anaemia type I**
D. Menike and S. N. Wickramasinghe

SHORT REPORTS

- 831 **Congenital dyserythropoiesis with intererythroblastic chromatin bridges and ultrastructurally-normal erythroblast heterochromatin: a new disorder**
S. N. Wickramasinghe, Ruth L. Spearing
and G. R. Hill
- 835 **Activation of the human delta-globin gene promoter in primary adult erythroid cells**
Delia C. Tang and Griffin P. Rodgers
- 839 **R411C mutation of the ALAS2 gene encodes a pyridoxine-responsive enzyme with low activity**
Kazumichi Furuyama, Ritsuko Uno,
Akio Urabe, Norio Hayashi, Hiroyoshi Fujita,
Masao Kondo, Shigeru Sassa
and Masayuki Yamamoto

- 842 **Prevalence of the C282Y and H63D mutations in the HFE gene in patients with hereditary haemochromatosis and in control subjects from Northern Germany**
P. Nielsen, S. Carpinteiro, R. Fischer,
J. M. Cabeda, G. Porto and E. E. Gabbe

Bone marrow hypoplasia

SHORT REPORT

- 846 **The incidence and significance of fevers during treatment with antithymocyte globulin for aplastic anaemia**
C. Dearden, T. Foukaneli, P. Lee,
E. C. Gordon-Smith and J. C. W. Marsh

Platelets, haemostasis and thrombosis

RESEARCH PAPERS

- 849 **Anti-CD36 autoantibodies in thrombotic thrombocytopenic purpura and other thrombotic disorders: identification of an 85 kD form of CD36 as a target antigen**
Duane R. Schultz, Patricia I. Arnold, Wenche Jy,
Peter A. Valant, Julie Gruber, Yeon S. Ahn,
Fang W. Mao, Wei W. Mao and Larry L. Horstman
- 858 **The P2Y₁ receptor, necessary but not sufficient to support full ADP-induced platelet aggregation, is not the target of the drug clopidogrel**
Béatrice Hechler, Anita Eckly,
Philippe Ohlmann, Jean-Pierre Cazenave
and Christian Gachet
- 867 **Plasminogen Kanagawa-I, a novel mutation, is caused by the amino acid substitution G732R**
Yumiko Higuchi, Kenichi Furihata, Ichiro Ueno,
Shinsuke Ishikawa, Nobuo Okumura,
Minoru Tozuka and Noriko Sakurai
- 871 **Additional genetic risk factors for venous thromboembolism in carriers of the factor V Leiden mutation**
Alberto Tosetto, Francesco Rodeghiero,
Ida Martinelli, Valerio De Stefano,
Edoardo Missiaglia, Patrizia Chiusolo
and Pier Mannuccio Mannucci
- 877 **Identification of new type 2B von Willebrand disease mutations: Arg543Gln, Arg 545Pro and Arg578Leu**
L. Hilbert, C. Gaucher, J. F. Abgrall,
A. Parquet, C. Trzeciak and C. Mazurier

SHORT REPORTS

- 885 **Two novel mutations (Pro864His, Val867Glu) causing type 2A von Willebrand disease and affecting a single restriction site in exon 28**
F. Bernardi, A. Casonato, G. Marchetti,
D. Gemmati, N. Bizzaro, E. Pontara
and A. Girolami

- 888 Factor V Arg³⁰⁶ → Thr (factor V Cambridge) and factor V Arg³⁰⁶ → Gly mutations in venous thrombotic disease
R. F. Franco, F. H. Maffei, D. Lourenço, V. Morelli, I. A. Thomazini, C. E. Piccinato, M. H. Tavella and M. A. Zago

- 891 Acute liver failure induced by alcohol and paracetamol in an HCV-infected haemophiliac
M. Leach, M. Makris, D. C. Gleeson and F. E. Preston

Correspondence

- 894 Complete splenic embolization in the treatment of immune thrombocytopenic purpura
F. Martinez Lagares, F. Fernandez Fuertes, T. Hernandez Cabrero, J. Bosch Benitez, J. Gonzalez San Miguel, Y. Martin, M. Tapia Martin, F. Muñoz Montero, A. Viejo Llorente, A. Givica Perez and J. M. Diaz Cremades

- 896 Announcements

VOL. 103 NO. 4

DECEMBER-II 1998

Annotation

- 897 Bone disease in β -thalassaemia major
B. Wonke

Review

- 902 Bone disease in multiple myeloma
Peter I. Croucher and Jane E. Apperley

Red cells and iron

RESEARCH PAPERS

- 911 High prevalence of low bone mass in thalassaemia major
C. E. Jensen, S. M. Tuck, J. E. Agnew, S. Koneru, R. W. Morris, A. Yardumian, E. Prescott, A. V. Hoffbrand and B. Wonke
- 916 Evaluation of cardiac status in iron-loaded thalassaemia patients following bone marrow transplantation: improvement in cardiac function during reduction in body iron burden
Egidio Mariotti, Emanuele Angelucci, Alberto Agostini, Donatella Baronciani, Ernesto Sgarbi and Guido Lucarelli
- 922 Molecular basis of hereditary methaemoglobinaemia, types I and II: two novel mutations in the NADH-cytochrome b₅ reductase gene
Koichiro Higasa, Jun-Ichi Manabe, Toshitsugu Yubisui, Hideki Sumimoto, Parichat Pung-armritt, Voravarn S. Tanphaichitr and Yasuyuki Fukumaki

- 931 Kupffer cell staining by an HFE-specific monoclonal antibody: implications for hereditary haemochromatosis
Judy M. Bastin, Margaret Jones, Christopher A. O'Callaghan, Lisa Schimanski, David Y. Mason and Alain R. M. Townsend

- 942 Dyserythropoiesis and severe anaemia associated with malaria correlate with deficient interleukin-12 production
K. Mohan and M. M. Stevenson

- 950 A new sickle cell disease phenotype associating Hb S trait, severe pyruvate kinase deficiency (PK Conakry), and an α 2 globin gene variant (Hb Conakry)
Michel Cohen-Solal, Claude Préhu, Henri Wajcman, Claude Poyart, Josiane Bardakdjian-Michau, Jean Kister, Danielle Promé, Colette Valentin, Dora Bachir and Frédéric Galactéros

SHORT REPORT

- 957 *In vitro* antisickling activity of cromolyn sodium
Ahmedou B. K. Fall, Michèle Toppet, Alina Ferster, Pierre Fondu, Renée Vanhaelen-Fastré and Maurice Vanhaelen

Haemopoiesis

RESEARCH PAPERS

- 960 Regulation of human erythropoietin gene induction by upstream flanking sequences in transgenic mice
J. Köchling, P. T. Curtin and A. Madan
- 969 Role of vascular endothelial growth factor (VEGF) and placenta-derived growth factor (PIGF) in regulating human haemopoietic cell growth
Mariusz Z. Ratajczak, Janina Ratajczak, Bogdan Machalinski, Marcin Majka, Wojtek Marlicz, Anna Carter, Zbigniew Pietrzkowski and Alan W. Gewirtz
- 980 Characterization of the response of human bone marrow endothelial cells to *in vitro* irradiation
Marie-Hélène Gaugler, Claire Squiban, Marie Claraz, Karin Schweitzer, Babette Weksler, Patrick Gourmelon and Anne Van der Meer

Bone marrow failure

RESEARCH PAPER

- 990 Dyskeratosis Congenita (DC) Registry: identification of new features of DC
Stuart Knight, Tom Vulliamy, Adrian Copplestone, Eliane Gluckman, Philip Mason and Inderjeet Dokal

RESEARCH PAPERS

- 997 New alleles of the platelet glycoprotein Ib α gene
J. Corral, R. González-Conejero, M. L. Lozano,
J. Rivera and V. Vicente
- 1004 A new variant of Bernard-Soulier syndrome
characterized by dysfunctional glycoprotein (GP)
Ib and severely reduced amounts of GPIX and GPV
Patrizia Noris, Eloisa Arbustini,
Pierangelo Spedini, Simona Belletti
and Carlo Luigi Balduini
- 1014 Characterization of HIV1-specific antibodies
and HIV-1-crossreactive antibodies to platelets
in HIV-1-infected haemophiliac patients
Wah Kiam Chia, Victor Blanchette,
Meera Mody, J. Fraser Wright and John Freedman
- 1023 Differential effects of three radiographic contrast
media on platelet aggregation and degranulation:
implications for clinical practice?
S. Heptinstall, A. White, N. Edwards, J. Pascoe,
H. M. Sanderson, S. C. Fox and
R. A. Henderson
- 1031 Laboratory abnormalities
in thrombocytopenic purpura
G. Rock, John G. Kelton, Kenneth H. Shumak,
Noel A. Buskard, David M. C. Sutton and
W. Barrett Benny
- 1037 Prothrombotic changes in children with sickle cell
disease: relationships to cerebrovascular disease
and transfusion
R. Liesner, I. Mackie, J. Cookson, S. McDonald,
A. Chitolie, S. Donohoe, J. Evans, I. Hann and
S. Machin
- 1045 Prothrombin antigen levels in symptomatic
and asymptomatic carriers of the 20210A
prothrombin variant
Paolo Simioni, Daniela Tormene,
Davide Manfrin, Sabrina Gavasso, Sonia Luni,
Domenico Stocco and Antonio Girolami
- 1051 A domain mutations in 65 haemophilia A families
and molecular modelling of dysfunctional factor
VIII proteins
MiaoLiang Liu, Michael E. P. Murphy and Arthur
R. Thompson

SHORT REPORTS

- 1061 Efficacy of high-dose methylprednisolone
as a first-line therapy in adult patients
with idiopathic thrombocytopenic purpura
Önder Alpdoğan, Tülin Budak-Alpdoğan,
Siret Ratip, Tülin Firatli-Tuğlular,
Serpil Tanriverdi, Sami Karti,
Mahmut Bayik and Tevfik Akoğlu

- 1064 Restarting antiplatelet regulation in prosthetic heart
valve patients after intracranial haemorrhage:
a 2-year follow-up
A. C. Butler and R. C. Tait

- 1067 Symptoms of inherited factor V deficiency
in 35 Iranian patients
M. Lak, R. Sharifian, F. Peyvandi
and P. M. Mannucci

Haematological malignancy

RESEARCH PAPERS

- 1070 A randomized double-blind placebo-controlled
study with subcutaneous recombinant human
erythropoietin in patients with low-risk
myelodysplastic syndromes
Italian Cooperative Study Group for rHuEpo
in Myelodysplastic Syndromes
- 1075 'Low-risk' myelodysplastic syndrome is associated
with excessive apoptosis and an increased ratio of
pro- versus anti-apoptotic bcl-2-related proteins
J. E. Parker, K. L. Fishlock, A. Mijovic,
B. Czepulkowski, A. Pagliuca and G. J. Mufti
- 1083 Overexpression of lung-resistance protein
and increased P-glycoprotein function in acute
myeloid leukaemia cells predict a poor response
to chemotherapy and reduced patient survival
Anton G. Borg, Robert Burgess,
Linda M. Green, Rik J. Scheper and
John A. Liu Yin
- 1092 Acute and chronic arsenic poisoning associated
with treatment of acute promyelocytic leukaemia
Shang-Yi Huang, Cheng-Shyong Chang,
Jih-Luh Tang, Hwei-Fang Tien, Tsung-Li Kuo,
Shiu-Feng Huang, Yu-Tung Yao,
Wen-Chien Chou, Chih-Yuan Chung,
Chiu-Hwa Wang, Ming-Ching Shen and
Yao-Chang Chen
- 1096 Structural and functional analysis
of the cytidine deaminase gene in patients
with acute myeloid leukaemia
Jan K. Schröder, Christoph Kirch, Siegfried Seeber
and Jochen Schütte
- 1104 P230 BCR/ABL protein may be associated
with an acute leukaemia phenotype
Cedrik Haskovec, Carola Ponzetto, Jaroslav Polák,
Diego Maritano, Zuzana Zemanová, Anna Serra,
Kyra Michelová, Hana Klamová, Jaroslav Čermák
and Giuseppe Saglio
- 1109 BCR-ABL oncoprotein is expressed by platelets
from CML patients and associated with
a special pattern of CrkL phosphorylation
George J. A. ten Bosch, Jan H. Kessler,
Johanna Blom, Antonia M. Joosten,
Carlo Gambacorti-Passerini,
Cornelis J. M. Melief and Onno C. Leeksa

- 1116 **V_H gene analysis of B-cell lymphoma in children from north-western Iran**
Caroline J. Chapman, Dennis Wright, H. Pour Feizi, Zadi Davis and Freda K. Stevenson

- 1124 **Clonal disease of natural killer large granular lymphocytes in Taiwan**
Wen-Chien Chou, I-Ping Chiang, Jih-Luh Tang, Ih-Jen Su, Shang-Yi Huang, Yao-Chang Chen, Ming-Chi Liu, Fenn-Yu Lee, Chiu-Hwa Wang, Ming-Ching Shen, Sou-Ming Chuang and Hwei-Fang Tien

- 1129 **Elevated serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) closely reflect tumour burden in chronic B-lymphocytic leukaemia**
Ilse Christiansen, Christer Sundström and Thomas H. Tötterman

- 1138 **Detection of the t(2;5)-associated NPM/ALK fusion cDNA in peripheral blood cells of healthy individuals**
L. Trümper, M. Pfreundschuh, E. V. Bonin and H. Daus

- 1145 **Clinical utility of immunoglobulin heavy chain gene rearrangement identification for tumour cell detection in multiple myeloma**
Agneta Swedin, Stig Lenhoff, Tor Olofsson, Britt Thureson and Jan Westin

- 1152 **Interleukin-1 in multiple myeloma: producer cells and their role in the control of IL-6 production**
Valérie Costes, Marielle Portier, Zhao-Yang Lu, Jean-François Rossi, Régis Bataille and Bernard Klein

SHORT REPORTS

- 1161 **Absence of p53 deletions in bone marrow plasma cells of patients with monoclonal gammopathy of undetermined significance**
Jutta Ackermann, Petra Meidlinger, Niklas Zojer, Heinz Gisslinger, Heinz Ludwig, Heinz Huber and Johannes Drach

- 1164 **Elevated serum soluble Fas ligand in natural killer cell proliferative disorders**
Kei Kato, Koichi Ohshima, Shigehiko Ishibara, Keizo Anzai, Junji Suzumiya and Masahiro Kikuchi

Transplantation

RESEARCH PAPERS

- 1167 **The impact of antenatal and perinatal variables on cord blood haemopoietic stem/progenitor cell yield available for transplantation**
A. A. Shlebak, I. A. G. Roberts, T. A. Stevens, R. M. Szydlo, J. M. Goldman and M. Y. Gordon

- 1172 **Mobilization of primitive haemopoietic progenitor cells and stem cells with long-term repopulating ability into peripheral blood in mice by pegylated recombinant human megakaryocyte growth and development factor**
Yoshifumi Torii, Yuko Nitta, Hiromichi Akahori, Tomonori Tawara, Tomoaki Kuwaki, Kinya Ogami, Takashi Kato and Hiroshio Miyazaki

SHORT REPORT

- 1181 **Pre-treatment of peripheral blood progenitor cells with macrophage inflammatory protein-1 α induces prolonged survival of early progenitor cells over 6 weeks of long-term culture**
Dietmar Egger, Christine Günther, Werner Helbig and Elisabeth Schulze

Transfusion medicine

RESEARCH PAPER

- 1184 **The Fy^x phenotype is associated with a missense mutation in the Fy^b allele predicting Arg89Cys in the Duffy glycoprotein**
Martin L. Olsson, Jonathan S. Smythe, Carola Hansson, Joyce Poole, Gary Mallinson, Jeff Jones, Neil D. Avent and Geoff Daniels

Rapid Papers

- 1192 **Leukapheresis cells of patients with multiple myeloma collected after mobilization with chemotherapy and G-CSF do not bear Kaposi's sarcoma associated herpesvirus DNA**
Frauke Bellos, Friedrich W. Cremer, Edith Ehrbrecht, Hartmut Goldschmidt and Marion Moos
- 1198 **Thymus-independent T-cell differentiation *in vitro***
M. Sanchez, E. Alfani, G. Visconti, A. M. Passarelli, A. R. Migliaccio and G. Migliaccio

Correspondence

- 1206 **Hepatitis G virus in patients with Hodgkin's lymphoma**
Marcello Persico, Amalia De Renzo, Eliana Persico, Rosario Notaro, Roberto Torella and Bruno Rotoli
- 1207 **Failure to detect anti-HTLV-I antibody in a patient with adult T-cell leukaemia/lymphoma**
Shan-Shun Luo, Hideto Tamura, Norio Yukose, Kiyoyuke Ogata and Kazuo Dan
- 1208 **Management of resistance to lymphoblastoid interferon alpha in chronic myeloid leukaemia**
L. M. Barton and A. R. Green

or
g.
ed

1209 True spectrum of 14q32 translocations
in multiple myeloma
G. Pratt, J. A. L. Fenton, J. A. Proffitt,
A. C. Rawstron, F. E. Davies, J. A. Child and
G. J. Morgan

1210 Multiple myeloma: infusions of low doses of donor
lymphocytes are also effective in reversing disease
recurrence after bone marrow transplantation
Cecile du Toit and Nicolas Novitzky

1212 Low and medium dose spleen radiation therapy
are able to induce long-term responses in
splenic lymphoma with villous lymphocytes
A. El Weshi, V. Ribrag, T. Girinski, M. El Hajj,
F. Dhermain and C. Bayle

lls

1213 A soft tissue extranodal mass as an initial
manifestation of splenic lymphoma with
circulating villous lymphocytes
M^a Teresa Orero, Amparo Miguel-Sosa,
Estella Matutes, Alicia Miguel-Garcia,
Fernando Hernández, Andrew Wotherspoon,
Magdalena Sánchez, Ana Pérez, Jose Simón
and Felix Carbonell

1215 Announcements

1215 Corrigendum

1216 Author and Keyword Indexes to Volume 103

ce

ro

a

The P2Y₁ receptor, necessary but not sufficient to support full ADP-induced platelet aggregation, is not the target of the drug clopidogrel

BÉATRICE HECHLER, ANITA ECKLY, PHILIPPE OHLMANN, JEAN-PIERRE CAZENAVE AND CHRISTIAN GACHET INSERM U.311, ETSS, Strasbourg, France

Received 27 April 1998; accepted for publication 25 August 1998

Summary. Recently we showed that the P2Y₁ receptor coupled to calcium mobilization is necessary to initiate ADP-induced human platelet aggregation. Since the thienopyridine compound clopidogrel specifically inhibits ADP-induced platelet aggregation, it was of interest to determine whether the P2Y₁ receptor was the target of this drug. Therefore we studied the effects of clopidogrel and of the two specific P2Y₁ antagonists A2P5P and A3P5P on ADP-induced platelet events in rats. Although clopidogrel treatment (50 mg/kg) greatly reduced platelet aggregation in response to ADP as compared to untreated platelets, some residual aggregation was still detectable. In contrast, A2P5P and A3P5P totally abolished ADP-induced shape change and aggregation in platelets from both control and clopidogrel-treated rats. A2P5P and A3P5P (100 µM) totally inhibited the [Ca²⁺]_i rise induced by ADP (0.1 µM) in control and clopidogrel-treated platelets, whereas clopidogrel treatment had no effect. Conversely, the inhibition of adenylyl

cyclase induced by ADP (5 µM) was completely blocked by clopidogrel but not modified by A2P5P or A3P5P (100 µM). A3P5P (1 mM) reduced the number of [³³P]2MeSADP binding sites on control rat platelets from 907 ± 50 to 611 ± 25 per platelet. After clopidogrel treatment, binding of [³³P]2MeSADP decreased to 505 ± 68 sites per platelet and further decreased to 55 ± 12 sites in the presence of A3P5P (1 mM). In summary, these results demonstrate that the platelet P2Y₁ receptor responsible for the initiation of aggregation in response to ADP is not the target of clopidogrel. Platelets may express another, as yet unidentified, P2Y receptor, specifically coupled to the inhibition of adenylyl cyclase and necessary to induce full platelet aggregation, which could be the target of this drug.

Keywords: P2T, antiplatelet drug, P2 receptor, [³³P]2MeSADP binding.

Nucleotide receptors, the so-called P2 receptors, are classified into two main families depending on their structure: the P2X family comprising 'ionotropic' or ligand-gated ion channel receptors and the P2Y family composed of G-protein coupled or 'metabotropic' receptors (North & Barnard, 1997). These families each contain several subtypes (P2Y₁ to P2Y₁₁ and P2X₁ to P2X₇) which differ in molecular structure and pharmacologic profile (North & Barnard, 1997; Communi *et al.*, 1998). P2 receptors are involved in many physiological processes including control of vascular tone, neurotransmission and platelet aggregation (Bhagwat & William, 1997). The central role of adenosine 5'-diphosphate (ADP) as an aggregating agent (Hellem,

1960; Gaardner *et al.*, 1961), not only in the physiological processes of haemostasis but also in the development and extension of arterial thrombosis (Maffrand *et al.*, 1988), has been long established and makes ADP receptors of potential clinical importance. Platelet activation by ADP leads to rapid calcium entry and mobilization of intracellular calcium stores (Heermanskerk & Sage, 1994), inhibition of adenylyl cyclase (Macfarlane, 1987), shape change from discoid to spherical form, and aggregation (Macfarlane, 1987; Hourani & Hall, 1996; Mills, 1996; Gachet *et al.*, 1997).

In view of the recent advances in our knowledge of the receptors mediating the effects of ADP on platelets, the P2T receptor should now be considered as a pharmacologic concept rather than a molecular entity (Gachet *et al.*, 1997). Thus, it has been demonstrated that platelets exhibit a functional P2X₁ receptor, activated by αβMeATP (MacKenzie *et al.*, 1996; Vial *et al.*, 1997), which is responsible for

Correspondence: Dr C. Gachet, INSERM U.311, Etablissement de Transfusion Sanguine de Strasbourg, 10 rue Spielmann, BP No. 36, 67065 Strasbourg Cédex, France.

the rapid calcium entry induced by ADP, but this receptor does not seem to be involved in ADP-induced platelet shape change or aggregation (Vial *et al.* 1997). since $\alpha\beta$ MeATP does not promote these responses and since desensitization of the P2X₁ receptor does not modify ADP-induced aggregation (Gachet *et al.* 1997). Several recent publications have reported detection of the P2X₁ receptor protein in platelets by immunoblot analyses (Scase *et al.* 1998; Sun *et al.* 1998; Clifford *et al.* 1997; Jin *et al.* 1998). The P2Y₁ receptor is also expressed by platelets (Léon *et al.* 1997; Jin *et al.* 1998). This receptor, when heterologously expressed in Jurkat cells, specifically couples to $[Ca^{2+}]_i$ mobilization and exhibits a pharmacological profile identical to that of the unknown platelet ADP receptor, ADP and related compounds being full agonists and ATP and its derivatives competitive antagonists (Léon *et al.* 1997; Hechler *et al.* 1998b). Furthermore, the fact that adenosine-2'-phosphate-5'-phosphate (A2P5P) and adenosine-3'-phosphate-5'-phosphate (A3P5P), which are specific P2Y₁ antagonists (Boyer *et al.* 1996), inhibit ADP-induced $[Ca^{2+}]_i$ increases, shape change and aggregation shows that the P2Y₁ receptor is responsible for these effects in platelets (Jin *et al.* 1998; Hechler *et al.* 1998a). On the other hand, the fact that under conditions where A2P5P and A3P5P totally abolish ADP-induced platelet aggregation and calcium movements, these molecules do not affect ADP-induced adenylyl cyclase inhibition, has led to the hypothesis of another P2 receptor coupled to adenylyl cyclase (Daniel *et al.* 1998; Hechler *et al.* 1998a).

The thienopyridine compound ticlopidine and its derivative clopidogrel, two specific and potent inhibitors of ADP-induced platelet aggregation, are used clinically as anti-thrombotic drugs (Schrör, 1993). Ticlopidine and clopidogrel are nevertheless inactive *in vitro* and must be metabolized in the liver in order to acquire their anti-aggregatory properties (Herbert *et al.* 1993). These compounds inhibit ADP-induced aggregation of human and rat platelets, the effect of ADP on adenylyl cyclase in human (Gachet *et al.* 1990), rat and rabbit platelets (Defreyn *et al.* 1991) and G-protein activation (Gachet *et al.* 1992), whereas they do not block ADP-induced platelet shape change (Gachet *et al.* 1995) or calcium movements (Gachet *et al.* 1990). Clopidogrel also irreversibly reduces the number of binding sites for radiolabelled 2-methylthio-adenosine 5'-diphosphate (2MeSADP) on human (Mills *et al.* 1992) or rat platelets (Savi *et al.* 1994; Gachet *et al.* 1995) by approximately 70%, leaving the residual sites insensitive to treatment. However, the precise mechanism of action of thienopyridines is still unknown and the molecular target of these drugs has not yet been identified.

In the present work we addressed the question of whether the P2Y₁ receptor could be the target of clopidogrel, by studying the effects of this drug and of A2P5P and A3P5P on ADP-induced platelet events.

MATERIALS AND METHODS

Materials. Adenosine 5'-O-(1-thiotriphosphate) (Sp-isomer) (Sp-ATP α S) was from Boehringer (Mannheim, Germany). 2MeSADP and propranolol were from Research

Biochemicals Incorporated (Natick, U.S.A.) and ADP, A2P5P, A3P5P, thrombin, prostaglandin E₁ (PGE₁), adrenaline and essentially fatty acid free human serum albumin from Sigma (Saint Quentin-Fallavier, France). Fura-2/acetoxymethyl ester (fura-2/AM) was purchased from Calbiochem (Meudon, France) and the cyclic adenosine 3'-5'-monophosphate (cAMP) assay kit from Amersham (Les Ulis, France). Apyrase was purified from potatoes as previously described (Cazenave *et al.* 1983). Clopidogrel was provided by Sanofi Recherche (Toulouse, France) and [³²P]2MeSADP (31.45 MBq/mmol) by Du Pont NEN® (Le Blanc Mesnil, France) and the radioligand was found to be 97.3% pure. A2P5P and A3P5P were checked for purity by high-performance liquid chromatography (HPLC) analysis on a Partisil 10 μ SAX column (Interchrom, Interchim, Monluçon, France) eluted with a linear gradient of 0–1 M ammonium phosphate buffer, pH 3.8.

Platelet aggregation studies. Male Wistar rats aged 7–10 weeks and weighing 200–250 g were used in groups of five animals. Clopidogrel was solubilized in water and arabic gum and force-fed to the rats twice at a dose of 50 mg/kg, 24 h and 2 h before blood collection, whereas control rats received water and arabic gum in the same manner. This dose of 50 mg/kg is known to induce the maximal inhibitory effect of clopidogrel (Gachet *et al.* 1995). Blood was drawn from the abdominal aorta under Rompun (0.2 ml/kg) and Imalgene 1000 (1 ml/kg) anaesthesia, anticoagulated with sodium citrate (3.15%) and centrifuged at 1570 *g* for 2 min to obtain citrated platelet-rich plasma (cPRP). Platelet aggregation was measured in cPRP from control and clopidogrel-treated rats by a turbidimetric method at 37°C in a dual-channel Payton aggregometer (Payton Associates, Scarborough, Ontario, Canada). A 450 μ l aliquot of platelet suspension was stirred at 1100 r.p.m. and activated by addition of different agonists, in the presence of control buffer or A2P5P or A3P5P at varying concentrations, in a final total volume of 500 μ l. The extent of aggregation was estimated quantitatively by measuring the maximum curve height above baseline.

Preparation of washed rat platelets. Blood anticoagulated with acid-citrate-dextrose solution (1 volume of ACD for 6 blood volumes) was centrifuged at 1570 *g* for 2 min at 37°C, after which the upper PRP phase was removed and centrifuged at 1570 *g* for 15 min at 37°C. The platelet pellet was washed twice in Tyrode's buffer (137 mM NaCl, 2 mM KCl, 12 mM NaHCO₃, 0.3 mM NaH₂PO₄, 2 mM CaCl₂, 1 mM MgCl₂, 5.5 mM glucose, 5 mM Hepes, pH 7.3) containing 0.35% human serum albumin as previously described (Cazenave *et al.* 1983) and finally resuspended at 3×10^5 platelets/ μ l in the same buffer, in the presence of 0.02 U/ml of the ADP scavenger apyrase (adenosine 5'-triphosphate diphosphohydrolase, EC 3.6.1.5), a concentration sufficient to prevent desensitization of platelet ADP receptors during storage. This preparation of washed platelets was kept at 37°C and used for adenylyl cyclase and binding experiments.

$[Ca^{2+}]_i$ measurements. After the first wash, the platelet pellet was resuspended in Tyrode's buffer containing 0.35% human serum albumin but no calcium, at a concentration of about 7×10^5 platelets/ μ l. Platelets were loaded with 15 μ M

fura-2/AM for 45 min at room temperature in the dark, in the presence of 0.1 mM aspirin to prevent platelet activation through synthesis of thromboxane A_2 . The suspension was then centrifuged at 1570 *g* for 8 min and finally resuspended at room temperature at a concentration of 2×10^5 platelets/ μ l, in Tyrode's buffer containing 0.02 U/ml apyrase and 0.1% essentially fatty acid free human serum albumin but no calcium. Aliquots of fura-2-loaded control or clopidogrel-treated rat platelets were transferred to a 10 \times 10 mm quartz cuvette maintained at 37°C and fluorescence measurements were performed under continuous stirring, using a PTI Deltascan spectrophluorimeter (Photon Technology International Inc., Princetown, N.J., U.S.A.). The excitation wavelength was alternately fixed at 340 or 380 nm, fluorescence emission was determined at 510 nm and results were calculated as the fluorescence ratio (340/380 nm).

Measurement of adenylyl cyclase activity. A 450 μ l aliquot of washed platelets from control or clopidogrel-treated rats was stirred at 1100 r.p.m. in an aggregometer cuvette and the following reagents were added at 30 s intervals: 10 μ M $CaCl_2$, 100 μ M A2P5P, A3P5P or ATP α S and 5 μ M ADP or vehicle (Tyrode's buffer containing no Ca^{2+} or Mg^{2+}). After 1 min the reaction was stopped by addition of 50 μ l ice-cold 6 \times N perchloric acid. Perchloric acid extracts were centrifuged at 11 000 *g* for 5 min to eliminate protein precipitate and cyclic AMP was isolated from the supernatants as described by Khym (1975) using a mixture of triethylamine and heion (28/22, v/v). The upper aqueous phase was lyophilized and the dry residue dissolved in the buffer provided with the commercial radioimmunoassay kit for cyclic AMP.

[^{33}P]2MeSADP binding. 2MeSADP, an analogue of ADP substituted at position 2 of the purine ring, is a more potent (about 10-fold) platelet aggregating agent than ADP (Mills

1996). Binding of [^{33}P]2MeSADP to washed platelets from control or clopidogrel-treated rats was determined by incubation of [^{33}P]2MeSADP (0.1 nM, 200 000 cpm) with washed platelet suspensions (3×10^5 platelets/ μ l) for 5 min at 37°C, in the presence or absence of 1 mM A3P5P, in a final volume of 1 ml in 3 ml polypropylene tubes. Experiments were started by addition of washed platelets to the reaction mixture and carried out in triplicate. The reaction was terminated by dilution with 2 ml of buffer and rapid filtration through Whatman GF/C glass filters under vacuum, after which the tubes and filters were rinsed twice. Radioactivity of the platelets on the filters was measured by scintillation counting (Wallac 1409 counter, Turku, Finland). Non-specific binding, determined by incubation in the presence of 1 μ M unlabelled 2MeSADP, amounted to about 5% of the total binding. Saturation and displacement experiments were performed using a single concentration of [^{33}P]2MeSADP (0.1 nM) and increasing concentrations of appropriate unlabelled ligands.

Data analysis. The concentration producing 50% of the maximal effect (EC_{50}) and the apparent dissociation constants of inhibitors (pA_2) were calculated using the GraphPad software package (GraphPad, San Diego, Calif., U.S.A.). Binding data were analysed and plotted with the LIGAND program (Munson & Rodbard, 1980).

RESULTS

Platelet aggregation studies

ADP-induced aggregation in cPRP of platelets from clopidogrel-treated rats was greatly reduced as compared to control platelet aggregation (Fig 1A). However, at this dose (50 mg/kg), there persisted a residual aggregation response

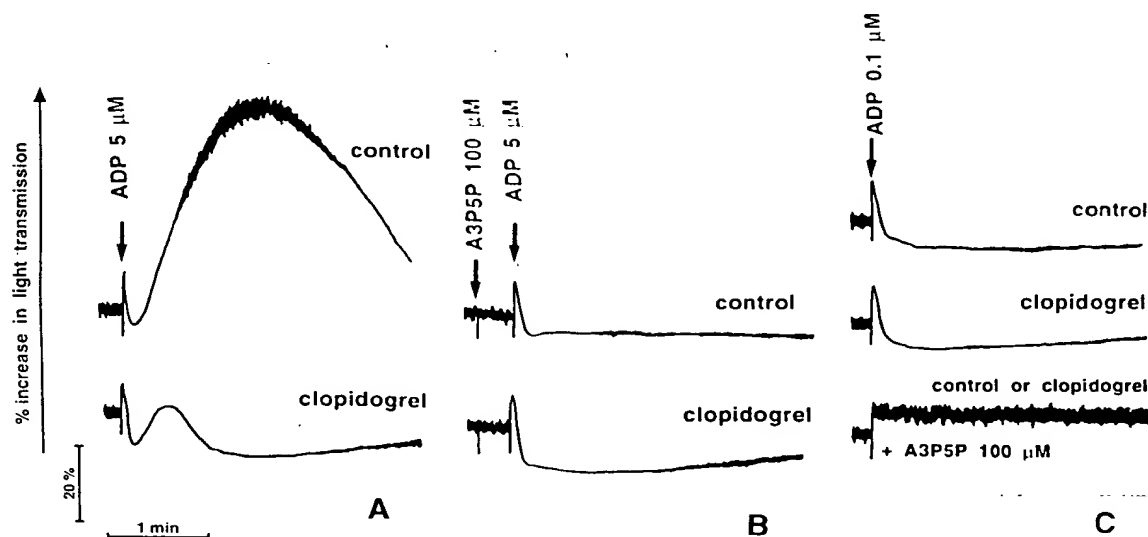


Fig 1. Effects of A3P5P on control and clopidogrel (50 mg/kg) treated rat platelets. (A) Aggregation in cPRP of platelets from control or clopidogrel-treated rats in response to 5 μ M ADP. (B) Aggregation induced by 5 μ M ADP was inhibited by 100 μ M A3P5P. (C) Shape change representative of five independent experiments giving identical results.

to 5 μM ADP. As already observed in previous work (Gachet *et al.*, 1990, 1995), clopidogrel did not influence the shape change induced by 0.1 μM ADP (data not shown). This is the primary morphological change of platelets which is reflected in a characteristic decrease in light transmission in the aggregometer. The adenine nucleotide derivatives A2P5P and A3P5P did not induce platelet shape change or aggregation, even at high concentrations (up to 100 μM). On the other hand, these two specific P2Y₁ receptor antagonists inhibited ADP-induced aggregation (Fig 1B) and likewise the shape change induced by 0.1 μM ADP (Fig 1C), in control or clopidogrel-treated rat platelets. A3P5P produced a parallel concentration-dependent shift to the right of the dose-response curve for ADP (Fig 2) in control platelets. EC₅₀ values for ADP-induced platelet aggregation were $1.6 \pm 0.2 \mu\text{M}$, $2.2 \pm 0.4 \mu\text{M}$, $2.6 \pm 0.7 \mu\text{M}$, $6.0 \pm 1.7 \mu\text{M}$, $11.7 \pm 2.8 \mu\text{M}$ and $19.2 \pm 2.1 \mu\text{M}$ in the presence of 0, 1, 3, 10, 30 and 100 μM A3P5P respectively. Schild analysis of the data resulted in a pA₂ value of 5.4 and a slope of 0.69 ± 0.05 , which suggests that the antagonism by A3P5P of ADP-induced rat platelet aggregation is non-competitive. The isomer A2P5P produced a similar right-hand shift of the dose-response curve for ADP and Schild analysis of the inhibition gave a pA₂ value of 5.6 and a slope of 0.74 ± 0.03 , also suggesting non-competitive antagonism.

[Ca²⁺]_i measurements

The ADP-induced intracellular calcium rise was not modified by clopidogrel treatment, in the presence (Figs 3A and 3C) or absence (Figs 3B and 3D) of 2 mM external calcium.

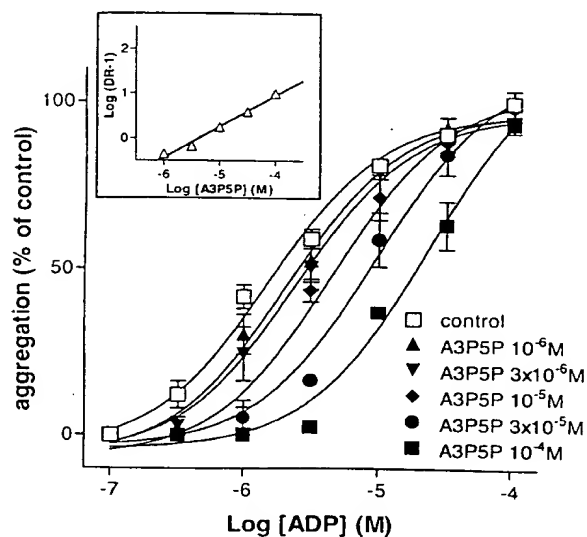


Fig 2. Inhibition by A3P5P of ADP-induced aggregation of control rat platelets. Aggregation was induced by increasing concentrations of ADP, alone or in the presence of increasing concentrations of A3P5P added 30 s before ADP. Inset: Schild regression analysis of the dose-response curve for ADP. Curves represent the mean of four independent experiments and bars show the SEM.

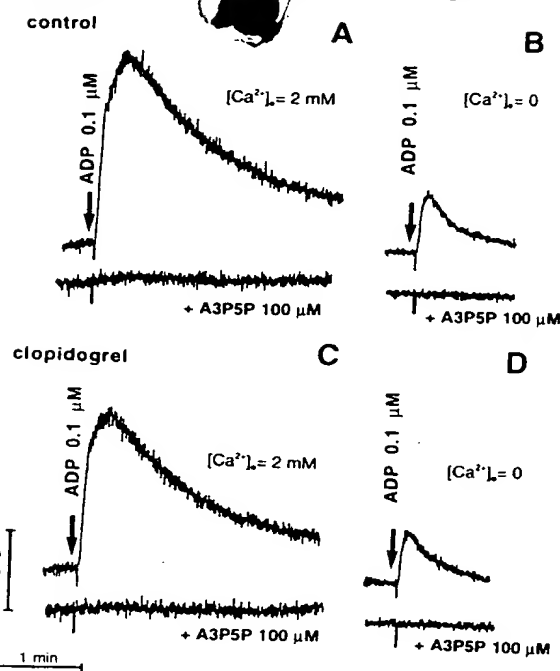


Fig 3. Effects of A3P5P (100 μM) and clopidogrel treatment (50 mg/kg) on [Ca²⁺]_i rises induced by 0.1 μM ADP. 100 μM A3P5P, added 30 s before ADP, totally abolished ADP-induced [Ca²⁺]_i increases in control (A, B) and clopidogrel-treated (C, D) rat platelets, in the presence of 2 mM external calcium (A, C) or in the absence of external calcium (0.2 mM EGTA) (B, D). Data are from one experiment representative of three independent experiments giving identical results.

Conversely, 100 μM A3P5P totally inhibited the [Ca²⁺]_i increases induced by 0.1 μM ADP, a concentration close to its EC₅₀ for this effect, in control or clopidogrel-treated platelets resuspended in Tyrode's buffer containing 0.35% human albumin and either 2 mM calcium (Figs 3A and 3C) or no calcium (0.2 mM EGTA) (Figs 3B and 3D). Identical inhibition of ADP-induced [Ca²⁺]_i rises was obtained using A2P5P (data not shown). These results suggest that platelet aggregation in response to ADP depended on the mobilization of internal calcium stores through activation of the P2Y₁ receptor and that this receptor was probably not the target of clopidogrel.

Adenyl cyclase activity

A2P5P and A3P5P (100 μM) had no influence on basal levels of cyclic AMP in rat platelets or on the cyclic AMP levels induced by 10 μM PGE₁ (data not shown). The inhibition by 5 μM ADP of PGE₁-stimulated cyclic AMP levels in control rat platelets was not reversed in the presence of 100 μM A3P5P or A2P5P, in contrast to 100 μM Sp-ATP α S, which totally reversed the effects of ADP (Fig 4, left panel). Sp-ATP α S is a well known antagonist of the ADP receptor affecting ADP-induced platelet aggregation, intracellular calcium increases

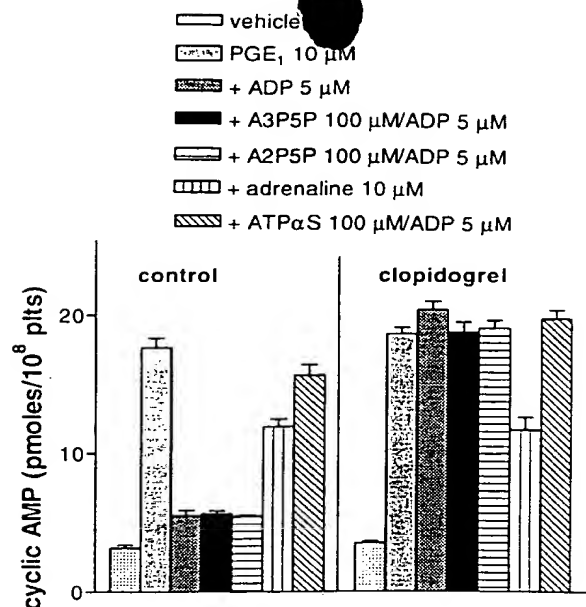


Fig 4. Cyclic AMP measurements in control (left panel) and clopidogrel (50 mg/kg) treated (right panel) washed rat platelets. Data are mean values (\pm SEM) from three separate experiments each performed in triplicate. When adrenaline was tested, the β -adrenergic specific antagonist propranolol (10 μ M) was added to avoid the β -adrenergic coupled stimulatory effect of adrenaline on adenylyl cyclase.

and adenylyl cyclase inhibition (Hourani & Hall, 1996; Mills, 1996). The α_2 coupled cyclase inhibitor adrenaline (10 μ M) was also able to inhibit PGE₁-stimulated cyclic AMP levels, provided its β -adrenergic coupled stimulatory effect on adenylyl cyclase was prevented by addition of the β -adrenergic-specific antagonist propranolol (10 μ M) (Fig 4, left panel).

ADP-induced adenylyl cyclase inhibition was, in contrast, completely abolished by clopidogrel treatment (Fig 4, right panel). This effect was specific, since 10 μ M adrenaline still inhibited PGE₁-stimulated cyclic AMP levels, which confirmed earlier observations that clopidogrel is a specific antagonist of ADP-induced inhibition of the adenylyl cyclase pathway.

Potentialiation by adrenaline of ADP-induced platelet aggregation

The aggregation of control or clopidogrel-treated rat platelets induced by 0.25 μ M ADP (Fig 5A, top and bottom, respectively) was potentiated in the presence of 10 μ M adrenaline (Fig 5C, top and bottom, respectively). However, under conditions where 100 μ M A3P5P totally inhibited ADP-induced aggregation (Fig 5B, top and bottom, respectively), no potentiation by adrenaline could be detected in either control or clopidogrel-treated platelets (Fig 5D, top and bottom, respectively), suggesting that the P2Y₁ receptor is necessary for the initiation of aggregation in response to ADP.

[³²P]2MeSADP binding

Saturation experiments using control rat platelets revealed 907 ± 50 [³²P]2MeSADP binding sites per platelet with an

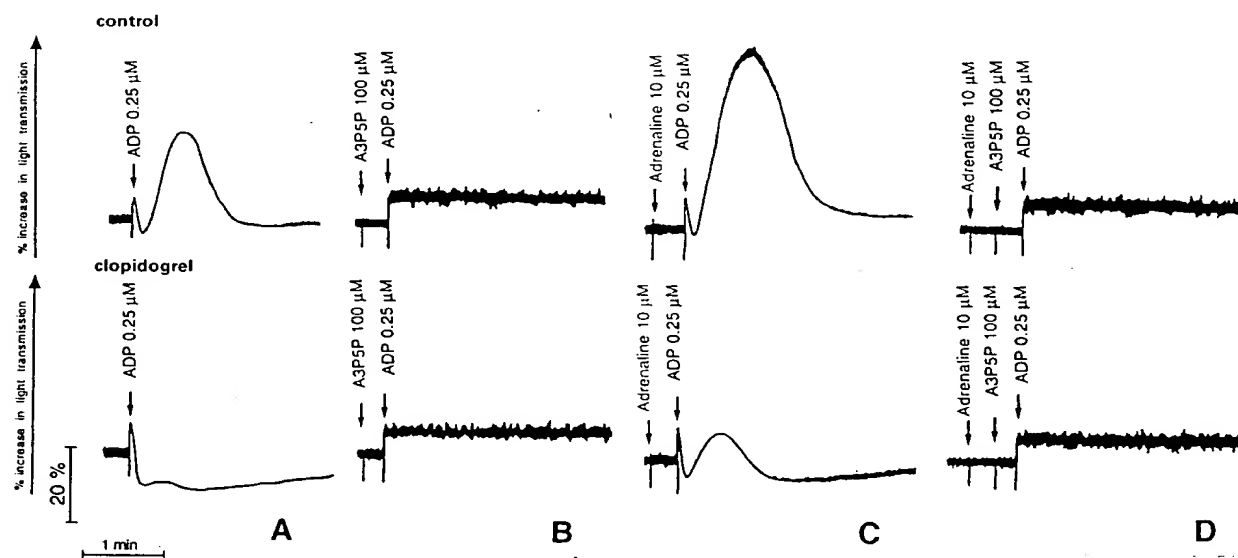


Fig 5. Potentiating effect of adrenaline on ADP-induced aggregation of platelets from control or clopidogrel-treated rats. Aggregation induced by 0.25 μ M ADP (A) was potentiated in the presence of 10 μ M adrenaline (C). This aggregation was inhibited by 100 μ M A3P5P in the absence (B) or presence (D) of 10 μ M adrenaline. Experiments were performed in the presence of the β -adrenergic-specific antagonist propranolol (10 μ M) to avoid the β -adrenergic coupled stimulatory effect of adrenaline on adenylyl cyclase. Results are from one experiment representative of three independent experiments giving identical results.

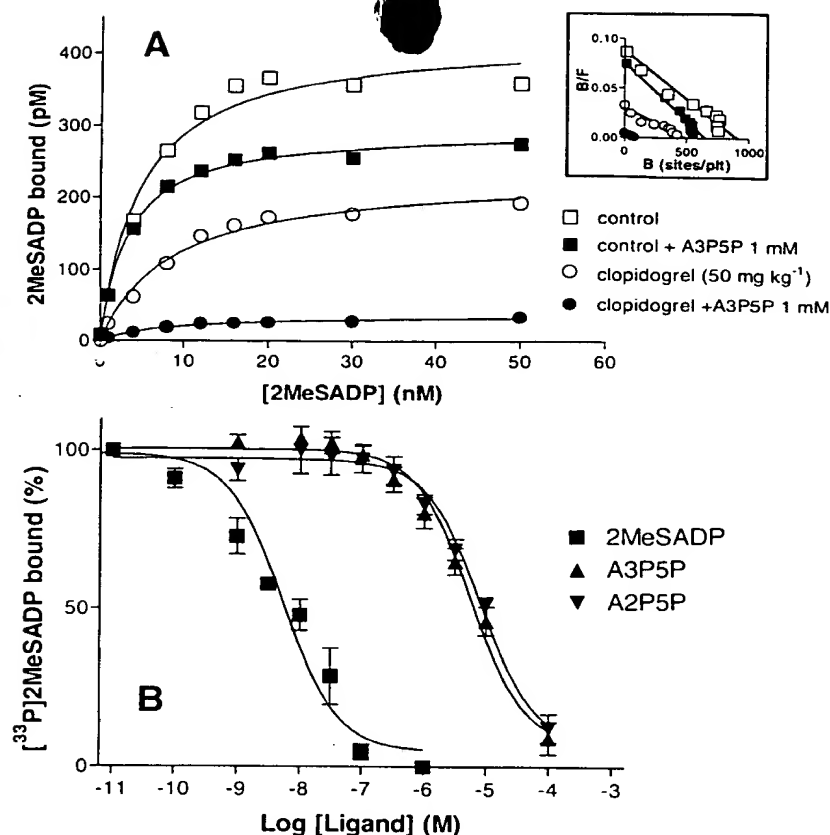


Fig 6. Binding of [³³P]2MeSADP to control and clopidogrel (50 mg/kg) treated washed rat platelets. (A) Equilibrium specific binding of [³³P]2MeSADP to control and clopidogrel-treated rat platelets in the presence or absence of 1 mM A3P5P. Data are mean values from one experiment performed in triplicate and are representative of four separate experiments giving identical results. (B) Competition curves for [³³P]2MeSADP binding to clopidogrel-treated rat platelets. Residual binding of [³³P]2MeSADP (0.1 nM) to clopidogrel-treated platelets was displaced by increasing concentrations of unlabelled 2MeSADP ($K_i = 8 \pm 1.3$ nM), A2P5P ($K_i = 9.5 \pm 1.1$ μ M) or A3P5P ($K_i = 8.7 \pm 1.2$ μ M). Points represent the mean values of nine data points.

affinity of 4.5 ± 0.3 nM (Fig 6A). In the presence of 1 mM A3P5P, the number of [³³P]2MeSADP binding sites decreased to 611 ± 25 per platelet and these residual sites displayed an affinity of 3.5 ± 0.2 nM. After clopidogrel treatment, binding of [³³P]2MeSADP was reduced by 50% as compared to control rat platelets, the remaining 505 ± 68 'clopidogrel insensitive' sites having an affinity of 8.3 ± 0.1 nM. Finally, in the presence of 1 mM A3P5P, [³³P]2MeSADP binding to clopidogrel-treated platelets fell to 55 ± 12 sites per platelet with an affinity of 5.9 ± 1.1 nM.

The residual binding of [³³P]2MeSADP to platelets from clopidogrel-treated rats was displaced by increasing concentrations of A2P5P ($K_i = 9.5 \pm 1.1$ μ M) or A3P5P ($K_i = 8.7 \pm 1.2$ μ M) and likewise by unlabelled 2MeSADP ($K_i = 8 \pm 1.3$ nM) (Fig 6B). These results suggest that the 'clopidogrel insensitive' sites could correspond to the P2Y₁ receptor.

DISCUSSION

In a recent study we demonstrated that the P2Y₁ receptor was necessary to initiate ADP-induced aggregation of human platelets (Hechler *et al.* 1998a), thus confirming and extending previous work which had shown the P2Y₁ receptor to be an ADP receptor present in platelets (Léon *et al.* 1997; Hechler *et al.* 1998b) and responsible for ADP-induced calcium mobilization and platelet shape change (Jin

et al. 1998). Since the thienopyridine compound clopidogrel specifically inhibits ADP-induced platelet aggregation, it was of further interest to determine whether the P2Y₁ receptor could be the target of this drug. Therefore in the present work we studied the effects of clopidogrel and of the two specific P2Y₁ antagonists A2P5P and A3P5P on ADP-induced platelet events.

When platelet aggregation was measured in citrated plasma, clopidogrel treatment led to an important reduction in ADP-induced aggregation as compared to untreated platelets, but a residual response was still detectable. These results confirm earlier observations that although clopidogrel is a specific inhibitor of ADP-induced platelet aggregation, part of the response to ADP is insensitive to this drug and in particular platelet shape change (Gachet *et al.* 1997). In contrast, ADP-induced shape change and aggregation of control rat platelets were totally inhibited by the two specific P2Y₁ antagonists A2P5P and A3P5P. This antagonism was selective, as A2P5P and A3P5P (100 μ M) did not affect platelet aggregation induced by thrombin (0.1 U/ml) under conditions where ADP secreted from platelet-dense granules was removed by addition of apyrase (0.2 U/ml) (data not shown). These results provide further evidence that the P2Y₁ receptor is necessary for the initiation of aggregation in response to ADP, and the fact that the inhibition by A2P5P and A3P5P was non-competitive reinforces the hypothesis

that ADP-induced aggregation involve more than one receptor. In addition, since residual aggregation of clopidogrel-treated rat platelets in response to ADP was completely inhibited by the two P2Y₁ antagonists, this receptor does not appear to be the target of clopidogrel.

At the intracellular level, A2P5P and A3P5P totally inhibited ADP-induced [Ca²⁺]_i mobilization but had no effect on ADP-induced inhibition of adenylyl cyclase. This supports the hypothesis that initiation of aggregation in response to ADP is dependent on mobilization of internal calcium stores and inhibition of adenylyl cyclase cannot alone promote aggregation (Haslam, 1973; Hechler *et al.* 1998a). The mechanism by which ADP induces the mobilization of intracellular calcium stores in platelets has been poorly understood due to controversy concerning the ability of ADP to activate phospholipase C (PLC) (Hourani & Hall, 1996; Mills, 1996; Gachet *et al.* 1997). Several studies suggested that this process could be independent of PLC activation (Vickers *et al.* 1990; Raha *et al.* 1993), whereas others found some inositol-1,4,5-triphosphate (IP3) formation in response to ADP stimulation (Daniel *et al.* 1986, 1998). Recently it has been possible to obtain knock-out mice lacking the gene

coding for the α subunit of the Gq protein (Offermans *et al.* 1997). Platelets from these mice did not aggregate in response to ADP under conditions where IP3 generation was totally abolished, which suggests that the PLC pathway is necessary for ADP stimulation to raise [Ca²⁺]_i and that this [Ca²⁺]_i increase is essential to platelet aggregation. However, since clopidogrel has no effect on the [Ca²⁺]_i rise induced by ADP but nevertheless strongly inhibits aggregation, an increase in [Ca²⁺]_i cannot solely account for platelet aggregation and inhibition of adenylyl cyclase, which is totally blocked under clopidogrel treatment, may be necessary to sustain a full aggregation response. Thus, the adenylyl cyclase pathway would potentiate the aggregation initiated by agonists of the P2Y₁ receptor. This potentiation of aggregation by inhibition of adenylyl cyclase is clearly demonstrated by the fact that adrenaline, which is specifically coupled through platelet α_2 -adrenergic receptors to adenylyl cyclase inhibition in the absence of any increase in [Ca²⁺]_i, does not itself induce platelet aggregation (Lanza *et al.* 1988) but potentiates the aggregation induced by ADP in control or clopidogrel-treated platelets. As no potentiation could be detected in the presence of 100 μ M A3P5P, a

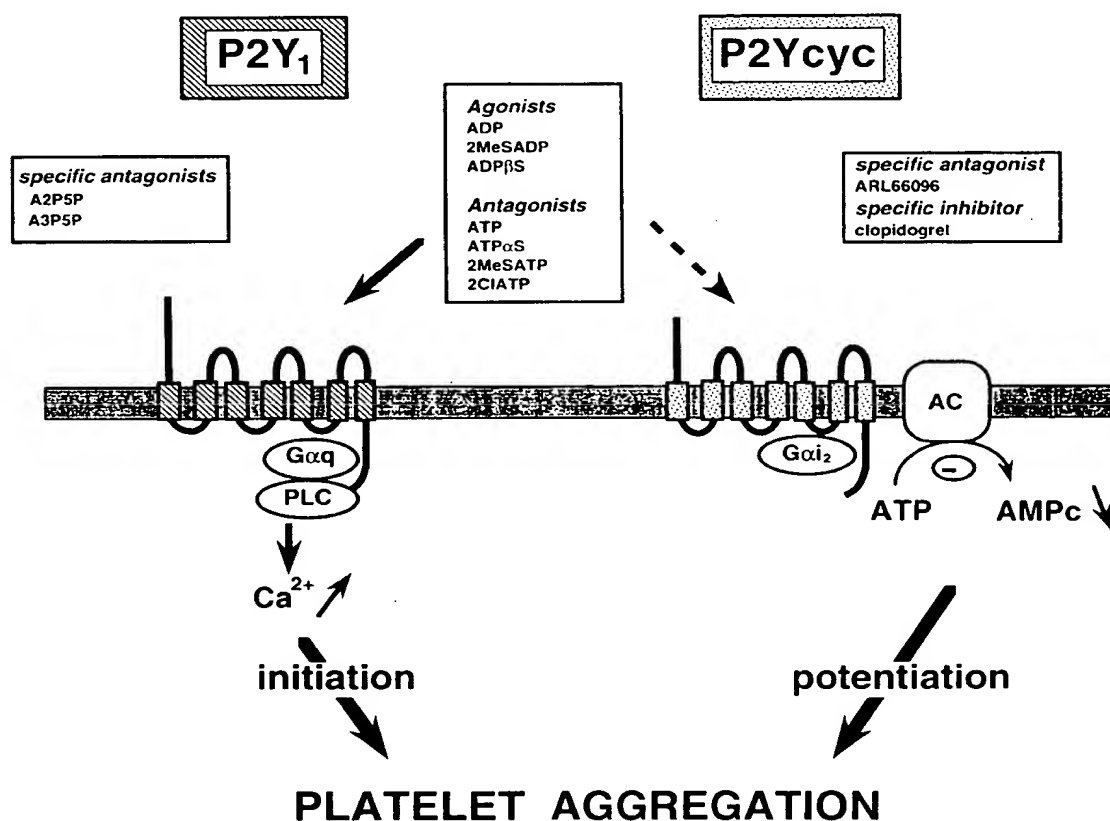


Fig 7. Current model of the mechanism of ADP-induced platelet aggregation. ADP initiates platelet aggregation through activation of the P2Y₁ receptor specifically coupled to the mobilization of internal calcium stores. Another, as yet unidentified, P2Y receptor, which we propose to call 'P2Y_{cyc}', specifically coupled to the inhibition of adenylyl cyclase, may potentiate the aggregation initiated by activation of P2Y₁. Specific antagonists of the P2Y₁ receptor include A2P5P and A3P5P. ARL66096 is a specific antagonist of the ADP-induced adenylyl cyclase pathway, whereas clopidogrel is a specific inhibitor and is used clinically as an antithrombotic drug.

concentration which totally abolishes ADP-induced aggregation of platelets from both control and clopidogrel-treated rats. Inhibition of adenylyl cyclase is clearly not sufficient to trigger this process. Therefore activation of the G_i pathway is clearly involved in the potentiation of platelet responses to ADP. However, since ADP does not induce a decrease in cyclic AMP in the course of aggregation, cyclic AMP levels cannot be directly responsible for this effect and further investigations will be required to define the mechanism of potentiation.

Binding experiments were performed to determine whether the P2Y₁ receptor was the molecular target of clopidogrel. Previous experiments using clopidogrel-treated rat platelets had distinguished two populations of 2MeSADP binding sites, 'clopidogrel sensitive' and 'insensitive' sites representing respectively 70% and 30% of the total binding sites (Savi *et al.* 1994; Gachet *et al.* 1995). In the present work we found that A3P5P inhibition led to a 30% reduction in the number of [³³P]2MeSADP binding sites on control platelets, whereas the sites insensitive to clopidogrel treatment amounted to about 50% of the initial binding sites present on control platelets. The proportion of clopidogrel-insensitive sites observed in these experiments was greater than in previous studies (Savi *et al.* 1994; Gachet *et al.* 1995). This discrepancy may be due to the fact that the earlier experiments were performed in female rats, whereas the present results were obtained in males, which have been shown to be less sensitive to clopidogrel treatment than females, even at high doses (Herbert *et al.* 1993). A3P5P almost totally abolished binding of [³³P]2MeSADP to the residual sites on clopidogrel-treated rat platelets, suggesting that the P2Y₁ receptor could represent about 30–50% of the total platelet ADP receptors and in fact correspond to the clopidogrel-insensitive binding sites.

The present study demonstrated that the target of clopidogrel is not the P2Y₁ receptor but the ADP-induced adenylyl cyclase pathway, thus reinforcing the hypothesis of the existence of a receptor distinct from P2Y₁ and specifically coupled to the inhibition of adenylyl cyclase. Although the P2Y₁ receptor would appear to be responsible for the initiation of aggregation, this receptor coupled to adenylyl cyclase, which we propose to call 'P2Y₁cyc', could mediate its further amplification (Fig 7). Identical conclusions were recently drawn using another P2Y₁ antagonist, A3P5PS, in rabbit platelets (Savi *et al.* 1998) and using A2P5P and A3P5P in human platelets (Hechler *et al.* 1998a; Jin *et al.* 1998; Daniel *et al.* 1998).

Daniel *et al.* (1998) have shown that ARL66096, like clopidogrel, is a specific and potent inhibitor of ADP-induced human platelet aggregation (Humphries *et al.* 1994), which reverses ADP-induced adenylyl cyclase inhibition but fails to inhibit ADP-induced platelet shape change, [Ca²⁺]_i mobilization or IP₃ production (Daniel *et al.* 1998). It is well established that ADP, which is stored at very high concentrations in platelet-dense granules, contributes to and reinforces aggregation in response to agents inducing dense granule secretion and hence plays a pivotal role in thrombosis (Maffrand *et al.* 1988). Clopidogrel inhibits the response to low concentrations of all aggregating agents

triggering platelet-dense granule secretion by blocking amplification of the aggregation process by released ADP. This drug has been found to be efficient in preventing the occurrence of thrombotic events in several cardiovascular pathologies (Herbert *et al.* 1993). ARL67085, a compound from the same series as ARL66096 (Humphries *et al.* 1995b), has shown antithrombotic activity in rat models and is under clinical investigation (Humphries *et al.* 1995a; Williams, 1996). It can be speculated that the potent antithrombotic effects of all these compounds originate from their inhibition of the ADP-induced adenylyl cyclase pathway.

In conclusion, our results show that the P2Y₁ receptor responsible for the mobilization of intracellular calcium stores and the initiation of platelet aggregation in response to ADP is not the target of clopidogrel. This target, which should be a P2Y receptor specifically coupled to adenylyl cyclase inhibition and responsible for amplification of the response initiated through P2Y₁, remains to be identified.

ACKNOWLEDGMENTS

The authors thank D. Cassel for expert technical assistance and J. N. Mulvihill for reviewing the English of the manuscript.

REFERENCES

- Bhagwat, S.S. & William, M. (1997) P2 purine and pyrimidine receptors: emerging superfamilies of G-protein-coupled and ligand-gated ion channel receptors. *European Journal of Medicinal Chemistry*, **32**, 183–193.
- Boyer, J.L., Romero-Avila, T., Schachter, J.B. & Harden, T.K. (1996) Identification of competitive antagonists of the P2Y₁ receptor. *Molecular Pharmacology*, **50**, 1323–1329.
- Cazenave, J.P., Hemmendinger, S., Beretz, A., Sutter-Bay, A. & Launay, J. (1983) L'agrégation plaquettaire: outil d'investigation clinique et d'étude pharmacologique. Méthodologie. *Annales de Biologie Clinique*, **41**, 167–179.
- Clifford, E.E., Parker, K., Humphreys, B.D., Kertesz, S.B. & Dubyak, G.R. (1998) The P2X₁ receptor, an adenosine triphosphate-gated cation channel is expressed in human platelets but not in human blood leukocytes. *Blood*, **91**, 3172–3181.
- Communi, D., Govaerts, C., Parmentier, M. & Boeynaems, J.M. (1998) Cloning of a human purinergic P2Y receptor coupled to phospholipase C and adenylyl cyclase. *Journal of Biological Chemistry*, **272**, 31969–31973.
- Daniel, J.L., Dangelmaier, C.A., Jin, J., Ashby, B., Smith, J.B. & Kunapuli, S.P. (1998) Molecular basis for ADP-induced platelet activation. I. Evidence for three distinct ADP receptors on human platelets. *Journal of Biological Chemistry*, **273**, 2024–2029.
- Daniel, J.L., Dangelmaier, C.A., Selak, M. & Smith, J.B. (1986) ADP stimulates IP₃ formation in human platelets. *FEBS Letters*, **206**, 299–303.
- Defrey, G., Gachet, C., Savi, P., Priot, F., Cazenave, J.P. & Maffrand, J.P. (1991) Ticlopidine and clopidogrel (SR 25990C) selectively neutralize ADP inhibition of PGE₁-activated platelet adenylyl cyclase in rats and rabbits. *Thrombosis and Haemostasis*, **65**, 186–190.
- Gardner, A., Jonsen, J., Laland, S., Hellem, A. & Owen, P.A. (1961) Adenosine diphosphate in red cells as a factor in adhesiveness of human blood platelets. *Nature*, **192**, 531–532.
- Gachet, C., Cattaneo, M., Ohlmann, P., Hechler, B., Lecchi, A.,

- Chevalier, J., Cassel, D., Manne, R.M. & Cazenave, J.P. (1995) Purinoceptors on blood platelets: further pharmacological and clinical evidence to suggest the presence of two ADP receptors. *British Journal of Haematology*, **91**, 434–444.
- Gachet, C., Cazenave, J.P., Ohlmann, P., Bouloux, C., Defreyn, G., Driot, F. & Maffrand, J.P. (1990) The thienopyridine ticlopidine selectively prevents the inhibitory effects of ADP but not of adrenaline on cAMP levels raised by stimulation of the adenylate cyclase of human platelets by PGE₁. *Biochemical Pharmacology*, **40**, 2683–2687.
- Gachet, C., Cazenave, J.P., Ohlmann, P., Hilf, G., Wieland, T. & Jakobs, K.H. (1992) ADP receptor-induced activation of guanine-nucleotide-binding proteins in human platelet membranes. *European Journal of Biochemistry*, **207**, 259–263.
- Gachet, C., Hechler, B., Léon, C., Vial, C., Leray, C., Ohlmann, P. & Cazenave, J.P. (1997) Activation of ADP receptors and platelet function. *Thrombosis and Haemostasis*, **78**, 271–275.
- Haslam, R.J. (1973) Interactions of the pharmacological receptors of blood platelets with adenylate cyclase. *Series Haematologica*, **6**, 333–350.
- Hechler, B., Léon, C., Vial, C., Vigne, P., Frelin, C., Cazenave, J.P. & Gachet, C. (1998a) The P2Y₁ receptor is necessary for ADP-induced platelet aggregation. *Blood*, **92**, 152–159.
- Hechler, B., Vigne, P., Léon, C., Breittmayer, J.P., Gachet, C. & Frelin, C. (1998b) ATP derivatives are antagonists of the P2Y₁ receptor: similarities with the platelet ADP receptor. *Molecular Pharmacology*, **53**, 727–733.
- Heermans, J.W.M. & Sage, S.O. (1994) Calcium signalling in platelets and other cells. *Platelets*, **5**, 295–316.
- Hellem, A.J. (1960) The adhesiveness of human blood platelets in vitro. *Scandinavian Journal of Clinical and Laboratory Investigation*, **12**, 1–117.
- Herbert, J.M., Frehel, D., Vallee, E., Kieffer, G., Gouy, D., Berger, Y., Nacciari, J., Defreyn, G. & Maffrand, J.P. (1993) Clopidogrel, a novel antiplatelet and antithrombotic agent. *Cardiovascular Drugs Review*, **11**, 180–198.
- Hourani, S.M.O. & Hall, D.A. (1996) P2T purinoceptors: ADP receptors on platelets. *P2 Purinoceptors: Localization, Function and Transduction Mechanisms*, pp. 53–70. Ciba Foundation Symposium 198. Wiley, Chichester.
- Humphries, R.G., Robertson, M.J. & Leff, P. (1995a) A novel series of P2T purinoceptor antagonists: definition of the role of ADP in arterial thrombosis. *Trends in Pharmacological Sciences*, **16**, 179–181.
- Humphries, R.G., Tomlinson, W., Clegg, J.A., Ingall, A.H., Kindon, N.D. & Leff, P. (1995b) Pharmacological profile of the novel P2T-purinoceptor antagonist, FPL 67085 in vitro and in the anaesthetized rat in vivo. *British Journal of Pharmacology*, **115**, 1110–1116.
- Humphries, R.G., Tomlinson, W., Ingall, A.H., Cage, P.A. & Leff, P. (1994) FPL 66096: a novel, highly potent and selective antagonist at human platelet P_{2T}-purinoceptors. *British Journal of Pharmacology*, **113**, 1057–1063.
- Jim, J., Daniel, J.L. & Kunapuli, S.P. (1998) Molecular basis for ADP-induced platelet activation. II. The P2Y₁ receptor mediates ADP-induced intracellular calcium mobilization and shape change in platelets. *Journal of Biological Chemistry*, **273**, 2030–2034.
- Khym, J.X. (1975) An analytical system for rapid separation of tissue nucleotides at low pressures on conventional anion exchangers. *Clinical Chemistry*, **21**, 1245–1252.
- Lanza, E., Beretz, A., Stierle, A., Hanau, D., Kubina, M. & Cazenave, J.P. (1988) Epinephrine potentiates human platelet activation but is not an agonist. *Agents. American Journal of Physiology*, **255**, 1276–1288.
- Léon, C., Hechler, B., Vial, C., Leray, C., Cazenave, J.P. & Gachet, C. (1997) The P2Y₁ receptor is an ADP receptor antagonized by ATP and expressed in platelets and megakaryoblastic cells. *FEBS Letters*, **403**, 26–30.
- Macfarlane, D.E. (1987) Agonists and receptors: adenosine diphosphate. *Responses and Metabolism* (ed. by H. Holmsen), pp. 19–36.
- MacKenzie, A., Mahaut-Smith, M.P. & Sage, S.O. (1996) Activation of receptor-operated cation channels via P2X₁ not P2T purinoceptors in human platelets. *Journal of Biological Chemistry*, **271**, 2879–2881.
- Maffrand, J.P., Bernat, A., Delebassee, D., Defreyn, G., Cazenave, J.P. & Gordon, J.L. (1988) ADP plays a key role in thrombogenesis in rats. *Thrombosis and Haemostasis*, **59**, 225–230.
- Mills, D.C. (1996) ADP receptors on platelets. *Thrombosis and Haemostasis*, **76**, 835–856.
- Mills, D.C., Puri, R.N., Hu, C.J., Minniti, C., Grana, G., Freedman, M., Colman, R.F. & Colman, R.W. (1992) Clopidogrel inhibits the binding of ADP analogues to the receptor mediating inhibition of platelet adenylate cyclase. *Atherosclerosis and Thrombosis*, **12**, 430–436.
- Munson, P.J. & Rodbard, D. (1980) LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Analytical Biochemistry*, **107**, 220–239.
- North, A. & Barnard, E.A. (1997) Nucleotide receptors. *Current Opinion in Neurobiology*, **7**, 346–357.
- Offermanns, S., Toombs, C.E., Hu, Y.H. & Simon, M.I. (1997) Defective platelet activation in Gαq-deficient mice. *Nature*, **389**, 183–186.
- Raha, S., Jones, G.D. & Gear, A.R.L. (1993) Sub-second oscillations of inositol 1,4,5-triphosphate and inositol 1,3,4,5-tetrakisphosphate during platelet activation by ADP and thrombin: lack of correlation with calcium kinetics. *Biochemical Journal*, **292**, 643–646.
- Savi, P., Beauverger, P., Labouret, C., Delfaud, M., Salet, V., Kaghad, M. & Herbert, J.M. (1998) Role of P2Y₁ purinoceptor in ADP-induced platelet activation. *FEBS Letters*, **422**, 291–295.
- Savi, P., Laplace, M.C., Maffrand, J.P. & Herbert, J.M. (1994) Binding of [³H]-2-methylthio-ADP to rat platelets: effects of clopidogrel and ticlopidine. *Journal of Pharmacology and Experimental Therapeutics*, **269**, 772–777.
- Sease, T.J., Heath, M.E., Allen, J.M., Sage, S.O. & Evans, R.J. (1998) Identification of a P2X₁ purinoceptor expressed on human platelets. *Biochemical and Biophysical Research Communications*, **243**, 525–528.
- Schrör, K. (1993) The basic pharmacology of ticlopidine and clopidogrel. *Platelets*, **4**, 252–261.
- Sun, B., Li, J., Okahara, K. & Kambayashi, J. (1998) P2X₁ purinoceptor in human platelets. *Journal of Biological Chemistry*, **273**, 11544–11547.
- Vial, C., Hechler, B., Léon, C., Cazenave, J.P. & Gachet, C. (1997) Presence of P2X₁ purinoceptors in human platelets and megakaryoblastic cell lines. *Thrombosis and Haemostasis*, **78**, 1500–1504.
- Vickers, J.D., Kinlough-Rathbone, R.L., Packham, M.A. & Mustard, J.E. (1990) Inositol phospholipid metabolism in human platelets stimulated by ADP. *European Journal of Biochemistry*, **193**, 521–528.
- Williams, M. (1996) Challenges in developing P2 purinoceptor-based therapeutics. *P2 Purinoceptors: Localization, Function and Transduction Mechanisms*, pp. 309–321. Ciba Foundation Symposium 198. Wiley, Chichester.